Feasibility and oncological safety of axillary reverse mapping in breast cancer, using premixed autologous serum and indocyanine green dye flourescence technique and an in-house near-infrared fluorescence imaging system and methylene blue dye.

***ARM** study*

A dissertation submitted in partial fulfilment of the requirements of

MS General Surgery (Branch - I) examination of the Tamil Nadu

Dr. MGR Medical University, Chennai to be held in March 2016.

Certificate

This is to certify that the dissertation entitled "***Feasibility and oncological safety of axillary reverse mapping in breast cancer, using premixed autologous serum and indocyanine green dye flourescence technique and an in-house near-infrared fluorescence imaging system and methylene blue dye***" is a bonafide work done by Jyothsna. M, MS General Surgery registrar in Division of Surgery at Christian Medical College, Vellore in partial fulfilment of the University rules and regulations for award of MS General Surgery under my guidance and supervision during the academic year 2012 to 2016.

Name & Signature

Guide	Head of Department	Principal
Dr. MJ Paul	Dr. John C Muthusami	Dr. Alfred Job Daniel
Professor	Head of Department	Principal
Endocrine Surgery	Division of Surgery	
CMC, Vellore	CMC, Vellore	CMC, Vellore

Declaration

This is to declare that this dissertation entitled "***Feasibility and oncological safety of axillary reverse mapping in breast cancer, using premixed autologous serum and indocyanine green dye flourescence technique and an in-house near-infrared fluorescence imaging system and methylene blue dye***" is a bonafide work done by Jyothsna. M, MS General Surgery registrar in Division of Surgery at Christian Medical College, Vellore in partial fulfilment of the University rules and regulations for award of MS General Surgery during the academic year 2012 to 2016.

Jyothsna . M

PG registrar,

MS General Surgery,

Division of Surgery,

CMC Vellore. 632004

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Surgical management options for patients with breast cancer vary from modified radical mastectomy, which includes axillary dissection for clinically axillary node positive patients to simple mastectomy with sentinel lymph node biopsy in cytologically assessed axillary node negative patients. Axillary surgery is associated with complications such paraesthesia over the arm and seroma formation, however lymphedema of the ipsilateral arm, remains as the most troublesome sequel of axillary dissection in patients with breast cancer.

Symptoms relief can be obtained to a certain extent by both manual and pneumatic devices, graded compression garments, pressure bandages, which aid in lymphatic drainage of the affected limb.(3)

Lymphedema predisposes the affected upper limb to repeated superficial soft tissue infections, leading to hospitalization and administration of antibiotics intravenously. It also causes discomfort and limits daily activities of the affected individuals.(2)

Lymphatic system

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Additional Vice Principal (Research)

April 09, 2013

Dr. M .Jyothsna
PG Registrar
Department of General Surgery III
Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project NEW PROPOSAL:**
To assess the feasibility and oncological safety of axillary reverse mapping in breast cancer, using indocyanine green flouresecene technique and in-house near-infrared flurescene imaging system.
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Ref: IRB Min. No. 8027 dated 01.10.2012

Dear Dr. M .Jyothsna,

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. M.J. Paul, Department of Endocrine Surgery, CMC

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Ref: IRB Min. No. 8027 dated 01.10.2012

Dear Dr. M .Jyothsna,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "To assess the feasibility and oncological safety of axillary reverse mapping in breast cancer, using indocyanine green flouresecene technique and in-house near-infrared flurescene imaging system" on October 1, 2012. I am quoting below the minutes of the meeting

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Proforma
3. Information Sheet and Informed Consent Form (English, Tamil and Hindi)
4. Cvs of Drs. M .Jyothsna, Inian Samarasam, M.J. Paul, Deepak Thomas Abraham, Marie Therese, Pooja Ramakant, Ms. Sypailyne Wankhar.
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The following Institutional Review Board (Research & Ethics Committee) members were present at the meeting held on October 1, 2012 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. Priya Abraham	MBBS, MD, PhD	Professor, Virology, CMC	Internal, Clinician
Dr. Srinivasa Babu	M.Sc, M.Phil, PhD	Sr. Scientist, Neurological Sciences, CMC	Internal, Scientist
Dr. Susanne Abraham	MBBS, MD	Professor, Dermatology, Venerlogy & Leprosy, CMC.	Internal, Clinician
Dr. Paul Ravindran	PhD, Dip RP, FCCPM	Professor, Radiotherapy, CMC	Internal,
Dr. Benjamin Perakath	MBBS, MS, FRCS	Professor, Surgery (Colorectal), CMC.	Internal, Clinician
Dr. B.J.Prashantham	MA (Counseling), MA (Theology), Dr Min(Clinical)	Chairperson(IRB)& Director, Christian Counselling Centre	External
Mrs. S. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Vathsala Sadan	M.Sc, Ph.D	Addl. Deputy Dean, College of Nursing, CMC.	Internal, Nurse
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M.Phil, BL.	Legal Advisor, CMC.	Internal, Legal Expert
Mr. Harikrishnan	BL	Lawyer, Vellore	External, Legal Expert
Mr. Sampath	BSc, BL	Advocate	External, Legal Expert
Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, DrPH(Epid), DMHC	Retired Professor, Vellore	External, Scientist
Mr. Joseph Devaraj	BSc, BD	Chaplain, CMC	Internal, Social Scientist

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Dr. Nihal Thomas	MD MNAMS DNB(Endo) FRACP(Endo) FRCP(Edin)	Secretary IRB (EC) & Dy. Chairperson (IRB), Professor of Endocrinology & Addl. Vice Principal (Research), CMC.	Internal, Clinician

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any serious adverse events occurring in the course of the project, any changes in the protocol and the patient information/informed consent. And on completion of the study you are expected to submit a copy of the final report.

A sum of Rs 40,000/- (Rupees Forty thousand only) will be sanctioned for 12 months. A subsequent installments of 40,000/- each will be released at the end of the first year following the receipt of the progress report (Total amount 80,000/- for 2 years).

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. M.J. Paul, Department of Endocrine Surgery, CMC

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

January 20, 2015

Ref: IRB - A1 - 12.01.2015

Dr. Jyothsna. M
PG Registrar
Department of General Surgery
Christian Medical College, Vellore 632 004

Ref: IRB Min. No. 8027 dated 1/10/12

Dear Dr. Jyothsna. M,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your revised objectives, inclusion criteria, exclusion criteria for the study titled: "To assess the feasibility and oncological safety of axillary reverse mapping in breast cancer, using premixed autologous serum and indocyanine green dye fluorescence technique and an in-house near-infrared fluorescence imaging system and methylene blue dye"

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on January 12th 2015 at 12.45 pm in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC	Internal, Clinician
Dr. Anand Zachariah	MBBS, PhD	Professor, Medicine, CMC	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC	Internal, Clinician
Dr. Niranjan Thomas	DCH, MD, DNB (Paediatrics)	Professor, Neonatology, CMC	Internal, Clinician

Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Jacob John	MBBS, MD	Associate Professor, Community health	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) D.M (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC	Internal, Clinician
Dr. Chandrasingh	MS, MCH, DMB	Professor, Urology, CMC.	Internal, Clinician
Dr. Anup Ramachandran	Ph. D	The Wellcome Trust Research Laboratory Gastrointestinal Sciences, CMC	Internal, Basic Medical Scientist
Dr. Simon Pavamani	MBBS, MD,	Professor, Radiotherapy, CMC.	Internal, Clinician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Dept. of Biostatistics, CMC.	Internal, Statistician
Dr. T. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Denise H. Fleming	B. Sc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMC	Internal, Scientist & Pharmacologist
Dr. Anuradha Rose	MBBS, MD	Assistant Professor, Community Health, CMC	Internal, Clinician

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel: 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

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Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC	Internal, Nurse
Mr. C. Sampath	BSc, BL	Legal Expert, Vellore	External, Legal Expert
Rev. Joseph Devaraj	B. Sc, BD	Chaplaincy Department, CMC	Internal, Social Scientist
Dr. Nihal Thomas	MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), Deputy Chairperson, IRB, Member Secretary (Ethics Committee), IRB	Internal, Clinician

We approve the above amendment as presented.

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD, MNAMS, DNB Endo, FRACP(Endo), FRCP(Edin), FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min. No. 8027 dated 1/10/12

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel: 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Acknowledgement

I thank God for his continual guidance and help at every step and for making this project a success.

I thank Dr. M. J. Paul, my teacher and guide for guiding me through this entire project and for his constant support and encouragement till the end.

I thank Dr. Syrpailyne W for her enthusiastic participation and scientific basis for her approach to the project.

I also thank Dr. Deepak Abraham, Dr. Pooja Ramakant, Dr. Anish Cherian and team for their valuable advice.

I also thank Mr. Bijesh Yadav from the department of Biostatistics for helping me with the statistical analysis

I thank my patients who willingly participated in the study, without whom this study would have been impossible.

Abstract

Title : Feasibility and oncological safety of axillary reverse mapping in early breast cancer, using premixed serum and indocyanine green dye flourescence technique and an in-house near-infrared fluorescence imaging system and methylene blue dye.

Department : Department of Endocrine Surgery and Bioengineering, Christian Medical College, Vellore – 632 004.

Name of the candidate: Jyothsna M

Degree and subject: MS General Surgery

Name of the Guide: M J Paul.

Objectives : To determine the metastatic rate and compare the detection rates of arm lymphatics and arm nodes, between serum and indocyanine green (ICG) dye, using an in-house near infrared (NIR) fluorescent imaging system and methylene blue dye, in patients with early breast cancer.

Methods: This IRB approved study included 52 patients with early breast cancer, undergoing ALND, equally allocated into two groups. In one group standardized solution of serum and ICG was injected intradermally posterior to the proximal part of the arm inter-muscular groove and in-house NIR imaging system was used and 2ml of methylene blue was injected at the same site in the other group. The identified ARM node is sent for histo-pathological examination to detect metastasis.

Results: After identifying the accurate site of injection, the identification rate of arm lymphatics and arm lymph node using serum and ICG and methylene blue were comparable. Metastatic rate in the arm node was low (5.8%). Thus ARM technique is feasible and safe in patients with early breast cancer.

Contents

Introduction:

Surgical management options for patients with breast cancer vary from modified radical mastectomy, which includes axillary dissection for clinically axillary node positive patients to simple mastectomy with sentinel lymph node biopsy in cytologically assessed axillary node negative patients. Axillary surgery is associated with complications such paraesthesia over the arm and seroma formation; however lymphedema of the ipsilateral arm, remains as the most troublesome sequel of axillary dissection in patients with breast cancer.

Symptom relief can be obtained to a certain extent by both manual and pneumatic devices, graded compression garments, pressure bandages, which aid in lymphatic drainage of the affected limb.(1)

Lymphedema predisposes the affected upper limb to repeated superficial soft tissue infections, leading to hospitalisation and administration of antibiotics intravenously. It also causes discomfort and limits daily activities of the affected individuals.(2)

Lymphatic system

Anatomy and physiology:

Human lymphatic system consists of three important components, first being an intricate and delicate network of capillaries which collect the extracellular fluid from different tissues and organs. Second, is a complex system of lymphatic channels and trunks which carry the lymph from the capillaries back in to the blood stream. And thirdly, lymph nodes which filter the lymph as it passes through them. *1*

Lymph is formed by the interstial fluid in the extracellular spaces, which is absorbed into the lymphatic capillaries. The diameter of lymphatic capillaries ranges from 10-50 microns and are lined by single layer endothelial cells, which lie on a basement membrane.

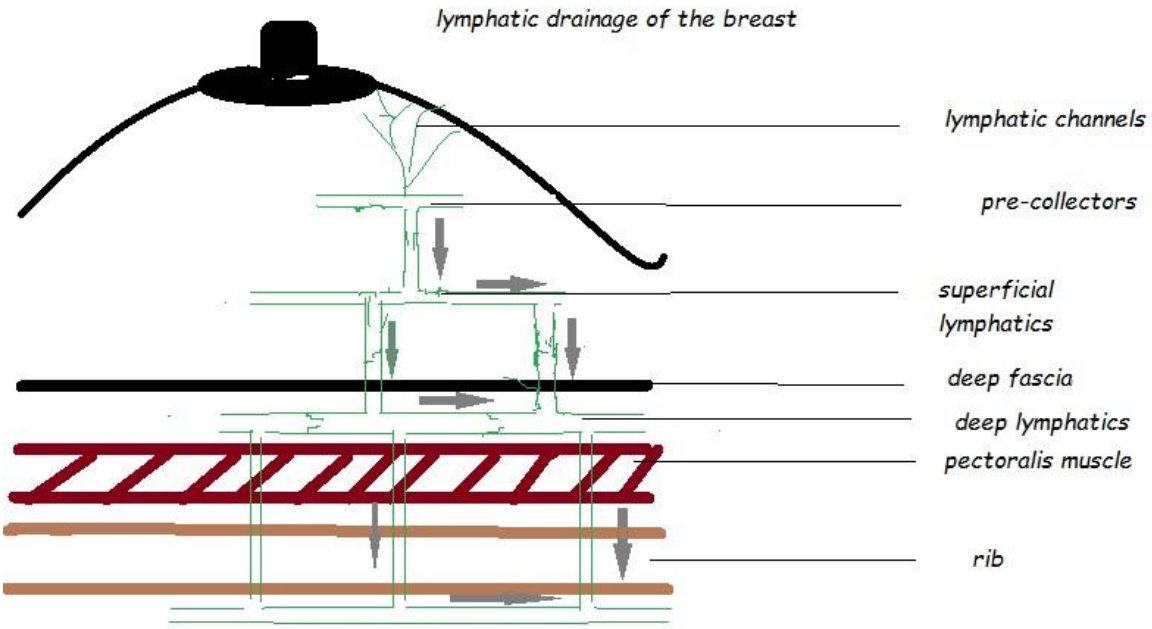
Endothelial cells overlap and these junctions function as valves and the allow the entry of small particles into the lymph. Collagen fibres prevent these capillaries from collapsing, by anchor them to the surrounding tissues.

Lymph is formed by diffusion and osmosis of extracellular fluid into the lymphatic capillaries. Inflow of lymph into the capillaries is regulated by osmotic gradient and by low luminal pressures caused by sequential contraction and forward propulsion of the lymph in the lymphatic channels.(3)

Composition of lymph is the similar to that of the plasma, with albumin constituting the majority. It is colourless and contains leucocytes, lymphocytes and few red blood corpuscles.

Lymphatic capillaries have many inter-capillary anastamoses which from plexuses and do not have valves. They form superficial and deep plexuses in the body. The superficial lymphatic plexus is smaller in calibre and communicate with deep plexuses at various places.

Lymphatic channels are distributed widely in the almost all parts of the body except in epidermis, cartilage, bone marrow, central nervous system. These lymphatic channels drain into larger calibre lymphatic vessels, which in turn drain into lymphatic trunks, that communicate with the venous system.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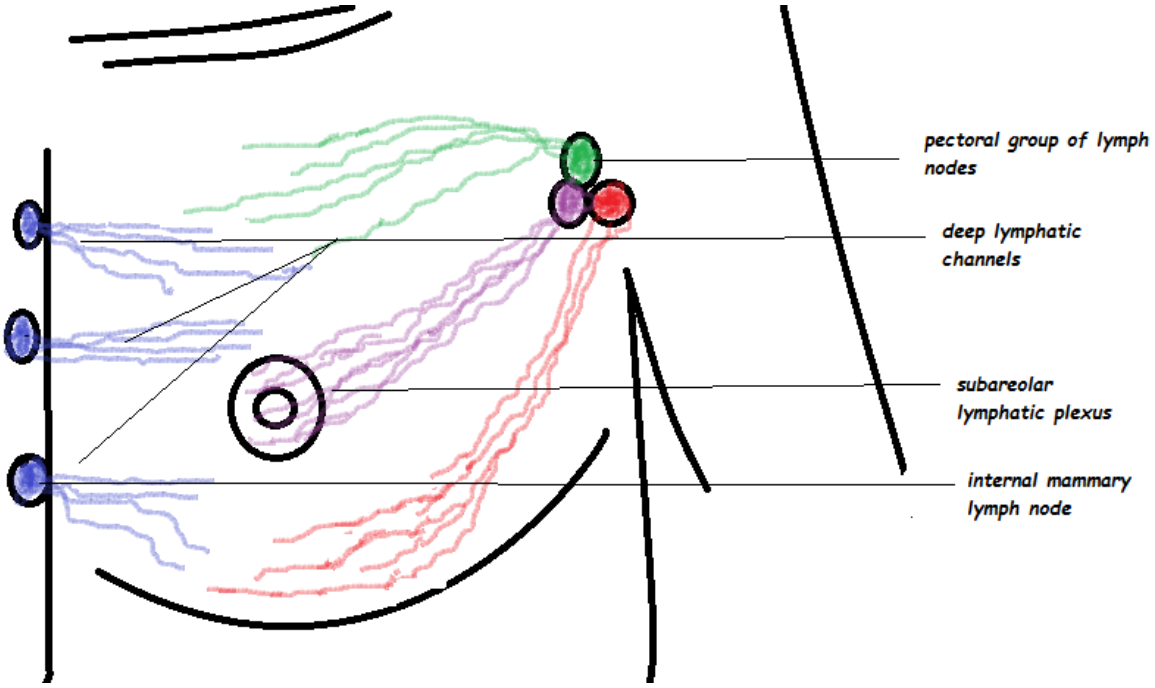


The wall of the lymphatic vessels consists of three layers.

1. Inner layer consists of thin, elongated, transparent endothelial cells which rest on the elastic basement membrane.
2. Middle layer consists of circularly arranged smooth muscle fibres and elastin fibres.
3. Outer layer consists of obliquely and longitudinally arranged smooth muscle and elastin fibres and connective tissue which attaches the lymphatic vessels to the adjacent tissues.(4)

Lymphatic flow in the tissues is determined by the rate of lymph formation, contraction of the circular and smooth muscle fibres in the wall of the lymphatic channels and forward propulsion of lymph and external pressures over the body, which can impede the lymphatic flow if they are prolonged at a one area. External pressure over the tissues can also enhance the lymphatic flow, when it is sequential and graded over a period of time.

Lymphatic flow is unidirectional, due to the presence of valves in the lymphatic vessels, which give a beaded appearance to them. These valves comprise of fibrous tissue, lined by endothelial cells on either sides and are similar to the valves that are present in the veins and are more in number in the lymphatic system. Smooth muscle fibres contract 10-15 times a minute and propels the lymph forward at a speed of 4-5 mm/sec. This peristalsis is regulated by filling pressure of 2-4 cm H20 and contraction, serotonin, prostaglandins and neural mechanisms. Lymphatic flow amounts to 2-4 L/day which varies according to physiological needs.

Lymph nodes are oval shaped, small glands that are situated along the course of the lymphatic channels and when they are not enlarged are usually indistinguishable from surrounding adipose tissue. Lymph nodes are covered with a capsule, which gives rise to incomplete fibrous septa that divide the gland into open spaces.

These spaces are filled with lymphoid tissue containing lymphocytes and sinuses which form a communication between the afferent and efferent vessels entering and exiting the gland, respectively, at the hilum.

Lymphatic system of the breast:

Sappey, first described the lymphatic drainage of the breast in 1870. The breast lymphatic drainage is separate from the underlying pectoralis muscles and chest wall.

It contains of a superficial lymphatic plexus which drain into the pectoral nodes, with a sub-areolar plexus which drains separately through the lymphatic channels into the pectoral or level I nodes in the axilla.

Lymphatic capillaries are present in the dermis and these do not have valves. The lymphatic capillaries drain into pre-collecting vessels, which have valves. The pre-collectors drain in the superficial lymphatic channels, which are present in the subcutaneous tissue. Superficial lymphatic channels drain in to the lymph nodes in the axilla. The superficial lymphatic channels drain into the deep lymphatics channels present below the deep fascia covering the pectoralis major muscle. These lymphatic channels drain into the axillary nodes.

Turner –warwick, in 1959 demonstrated that lymphatics of the breast drains separately into the axilla and not via sub-areolar plexus. There are perforating channels which connect the superficial and deep lymphatic channels. The deep lymphatic channels are present below the deep fascia. These perforating channels drain along the branches of internal mammary vessels and drain into the internal mammary nodes, which are situated along the internal mammary artery.(3)(5)

Axillary lymph nodes are divided into 6 groups

1. Anterior (pectoral) axillary nodes
2. Posterior (sub scapular) axillary nodes
3. Lateral (axillary vein) nodes
4. Central nodes
5. Apical nodes (sub clavicular)

   Interpectoral group (Rotter's lymph nodes)

   These lymph node groups are assigned to three levels according to their relationship with the pectoralis minor muscle.

Levels of axillary lymph nodes:

Level 1: includes lymph nodes which lie below the lower border of the pectoralis minor muscle

Level 2: includes lymph nodes which lie behind the pectoralis minor muscle.

Level 3: includes lymph nodes which lie above the upper border of pectoralis minor muscle and below the clavicle.

Surgical anatomy of the axilla:

Axilla is a pyramidal shaped space bound by four borders and has a base and an apex

Borders of the axilla:

Anterior border is formed by pectoralis major and minor muscles, along with subclavius muscle.

Posterior border is formed by teres minor, latissimus dorsi, subscapularis muscles.

Lateral border is formed by biccipital groove on the medial aspect of the humerus, on to which the anterior and posterior border muscles insert.

Base is formed by skin, subcutaneous tissue and axillary fascia.

Apex is formed by costo-clavicular ligament.

Medial border is formed by the ribs and the intercostal muscles along with the overlying serratus anterior muscle.

Contents of axilla:

Axillary pad of fat, which also contains the lymphatic channels and the axillary lymph nodes.

Thoracodorsal vein and the artery, that course along the thoraco-dorsal nerve, along the posterior border.

Long thoracic nerve, which courses along the medial border, over the serratus anterior muscle below the fascia and supplies the muscle.

Medial and lateral pectoral nerves, which supply the pectoralis major and minor muscles.

Intercosto-brachial nerves, which provide sensory supply to the arm and skin over the axilla.

Axillary vein and the axillary artery, along with the brachial plexus also form the contents .

Axillary lymph node dissection:

Surgical technique:

Pre operative: Informed consent has to be obtained prior to the procedure and side has to be marked correctly. All safety precautions for the patient have to be followed. Preoperative antibiotic should be administered thirty minutes prior to the procedure.

Anaesthesia: The procedure is performed under general anaesthesia without giving neuromuscular agents to the patient, as it helps in identification of the motor nerves and prevent their injury during dissection.

Preparation : The skin over the surgical field has to be prepared with alcohol/iodine based antiseptic solution. This should extend from the neck, supraclavicular region to the ipsilateral inframammary region, in the longitudinal plane. Transversely, skin should be prepared from the opposite nipple upto the ipsilateral posterior axillary line.

The ipsilateral arm should also be prepared circumferentially, up to the elbow and draped separately.

Incision:

In patients undergoing axillary dissection, an incision along the skin crease, 2 cm below the axillary hair line, is made to gain access into the axilla.

In patients who are undergoing modified radical mastectomy, axilla can be entered through the same incision after raising the skin flaps.

Dissection: Fatty tissue and lymph nodes below the axillary vein are cleared, taking care to avoid injury to axillary vein, thoracodorsal bundle and long thoracic nerve during dissection. Intercosto-brachial nerves can be identified and spared to prevent hyperasthesia over the medial aspect of the arm and skin over the axilla. Medial pectoral vessels are identified and preserved. Level III nodes can be cleared when there is a suspicion or when they are enlarged. Hemostasis should be achieved and drains have to placed under the flaps and in the axilla. Subcutaneous tissue closed with interrupted absorbable sutures and skin is closed with interrupted non-absorbable sutures.

Post-operative complications:

Hematoma : Postoperative hematoma is known to occur in 2-10% of patients despite the advent of electrocautery. Achieving good hemostasis is the key to prevent hematoma formation.

Seroma : Disruption of the lymphatic channels during dissection leads to lymph leak into the closed space, temporarily. This can be prevented by placing drains during the procedure and removing them once the output decreases. At times, seroma formation can occur after the drains are removed. In such situations, repeated aspiration can be done to evacuate the seroma, as it interferes with the wound healing. Patients also tolerate this procedure, due to the sensory loss over the flaps.

Wound infection: Its reported incidence ranges from 1-20%. Staphylococcal organisms are the most common causative agents. Predisposing factors such as obesity, diabetes mellitus and elderly age are common. Use of preoperative cephalosporins, administered intravenously, reduces the wound infection rates by 40%. (6)

When there is a associated abscess or an underlying seroma, aspiration and drainage must be performed. Minimal wound discharge and surrounding cellulitis can be treated with oral antibiotics. When there is a persistent pus discharge and increased area of redness, hospital admission and administration of intravenous antibiotics should be considered.

Lymphedema of the ipsilateral arm : it is one of the dreaded complication following axillary lymphnode dissection. Its incidence increases with the extent of axillary dissection. Most patients develop swelling of the ipsilateral arm with in 2 years and its incidence rises as duration from the time of surgery increases.

Lymphedema occurs due to disruption of the lymphatic channels that drain the arm, during axillary dissection. As a result, the lymph in the arm lymphatic channel accumulates and extravasates into the surrounding tissue causing inflammatory reaction and fibrosis. This leads to non-pitting edema, and swelling of the affected arm. Lymphedema causes significant morbidity in patients undergoing axillary dissection. And their day to day activities are limited due to pain, swelling and discomfort in the arm.

Incidence of lymphedema ranges from 0-13%, even in patients undergoing Sentinel lymph node biopsy (SLNB), a procedure which helps to avoid axillary dissection, so as to prevent secondary lymphedema, in breast cancer patients with no enlarged axillary lymphnodes. (7)(8) Lymphedema was found in 11-30% of patients undergoing ALND (9). The highest reported incidence is 56% (10)

In a prospective study conducted on a cohort of 103 patients who were undergoing mastectomy with axillary lymph node dissection for proven breast cancer, in Christian Medical College, Vellore, the incidence of lymphedema was found to be 25.24%.

Aims and Objectives

The aim of this study is to assess the feasibility and oncological safety of axillary reverse mapping (ARM) in early breast cancer.

The objectives of this study are

1. To determine and to compare the detection rate and location of the arm lymphatics and lymph node using two different techniques, a premixed solution of autologous serum and indocyanine green(ICG) dye and an in-house near infra-red fluorescent imaging system, and also by injecting 2 ml of methylene blue dye, posterior to the medial groove of the ipsilateral arm.

2. To determine the metastatic rate in the ARM node in breast cancer.

   The hypothesis is that the lymphatic pathway from the arm and the associated lymph node, may not be involved by metastasis from the primary breast cancer; therefore by preserving them secondary lymphedema may be prevented.

Literature review:

Axillary surgery is associated with complications such paraesthesia over the arm and seroma formation; however lymphedema of the ipsilateral arm, remains the most troublesome sequel following axillary dissection in breast cancer patients. Symptom relief can be obtained to a certain extent by both manual and pneumatic devices, graded compression garments, pressure bandages, which aid in lymphatic drainage of the affected limb.(1)

Lymphedema predisposes the affected upper limb to repeated superficial soft tissue infections, leading to hospital admissions and use of intravenous antibiotics. It also causes discomfort and limits daily activities of the affected individuals.(2)

Incidence of lymphedema ranges from 0-13%, even in patients undergoing Sentinel lymph node biopsy (SLNB), a procedure which helps to avoid axillary dissection, so as to prevent secondary lymphedema, in breast cancer patients with no enlarged axillary lymphnodes. (7)(8) Lymphedema was reported in 11-30% of patients undergoing ALND (9). The highest reported incidence is 56% (10)

In a prospective study conducted on a cohort of 103 patients who were undergoing mastectomy with axillary lymph node dissection for proven breast cancer, in Christian Medical College, Vellore, the incidence of lymphedema was found to be 25.24%.

Axillary reverse mapping (ARM) is a technique first described in 2007, to prevent lymphedema in patients undergoing ALND. This technique was based on the evidence, that lymphatic drainage of the breast was separate from lymphatic drainage of the upper limb, as described by Sappey(11) and Turner -Warwick(12) and the hypothesis that lymph nodes draining the arm were not involved in patients with breast cancer. In the sentinel lymph node biopsy technique, the lymphatic channels and lymph node draining the breast are identified.

Mapping the arm lymphatic channels and detection of arm draining lymph nodes and preserving them, may prevent lymphedema and the morbidity associated with it in patients undergoing axillary dissection, as anatomic continuity in arm lymphatic drainage is maintained. Potentially, this can save many more patients from the lymphedema complication than the sentinel node technique.

However, there were reports of metastasis in ARM node and cross-over between the SLN and ARM node.(13) And according to oncological principles it is not safe to leave behind the metastatically involved arm draining nodes during ALND.

ARM technique

It was first described using isosulphan blue dye by Suzanne Klimberg and team in Rockefeller Cancer Institute. After the induction of general anaesthesia, about 2-2.5 ml of blue dye was injected subcutaneously or intradermally, in the upper inner aspect of the medial groove between the triceps and biceps muscles of the ipsilateral arm. This site was chosen as it is closer to axilla, which aided in quick drainage in the lymphatic channels and detection of lateral group of lymph nodes and also the skin tattoo at the site of the injection will be hidden underneath the clothing. The injection site was massaged and arm was elevated, to aid in drainage of the blue dye in the lymphatic channels towards axilla.(14)(15). The blue arm nodes were identified and dissected from the axilla and sent separately for histo-pathological evaluation

The detection rates of blue arm lymphatic channels and arm nodes in patients undergoing axillary dissection, in various studies ranged from 40.6% to 91% (9)(10)(11)(16). Radioisotope (injected into the ipsilateral hand) was also used in combination with blue dye in increase the detection rates of arm node.(17)

Complications such as pain, blue staining of skin and persistence of staining for few months were reported, following ARM technique using blue dye.(18)

To increase detection rates and to reduce local complications caused by blue dye, radioisotopes and fluorescent dyes were used to trace the lymphatic channels. ARM technique, when performed using indocyanine green, a fluorescent dye and fluorescence imaging device, yielded 85% detection of arm lymphatic channels and arm node. (19)

Preliminary work:

Choosing between blue dye and fluorescence imaging techniques.

Lymphedema, secondary to axillary dissection is a major concern for most of our patients who are undergoing surgery for breast cancer. Incidence of lymphedema in patients undergoing the same procedure, is 25.24% at our institute. Axillary reverse mapping seems promising in preventing lymphedema in this group of patients. We wanted to assess the feasibility and safety of this technique, in our setting.

Initially we used methylene blue to map the arm lymphatic channels in few patients using the technique of intermuscular groove injection described earlier. But we were not able to detect the lymphatic channels or the arm node.

As the documented detection rates of arm lymphatic channels and arm lymph nodes using blue dye, in literature were low, we decided to use indocyanine green instead, which is a fluorescent dye to trace the lymphatic channels, after obtaining expert opinion from Dr. Suzanne Klimberg, Director of the Breast Cancer Program, Rockefeller Cancer Institute.

Fluorescence imaging:

Fluorescence imaging, is an efficient method of visualising cells, tissues and vasculature both invitro and invivo.(20)

Fluorescence imaging is superior to the current imaging modalities are that it has a good signal to noise ratio [SNR] making the area of interest visible in a dark background. It also has high sensitivity as lower concentrations of the fluorophore can be detected. And it provides good spatial and temporal information not only at molecular level but also of tissues and blood vessels and lymphatic channels.

Near infrared fluorophores are probes whose excitation and emission wavelengths are in the range of 745-850nm. These fluorophores can be excited by an simple NIR LED/laser and emit a fluorescent signal with reduced scattering and have a good tissue penetration.(21)

The common fluorescent dyes used are ICG, Cy 5.5, Cy7, Irdye800 CW, and ProSense 750. At present, Indocyanine green dye is the o only NIR fluorophore approved by US Food and Drug Administration(FDA).

Indocyanine green was first developed by Kodak research laboratories for NIR photography in 1955 and was approved for use in clinical applications in 1956(22)

Properties of Indocyanine green dye:

Its an anion, that belongs to the group of cyanine dyes. It's a tri-carbocyanine dye, with a molecular weight of 751.4Da. ICG in the dry form is stable at room temperature and it is available in this form in the pharmaceuticals. It is readily soluble in distilled water but not soluble in saline.(23) It is unstable in solutions (more than 10 hours) at room temperature and when exposed to light. It binds to the plasma lipoproteins and it does not alter the protein structures. It is does not have metabolites and it is excreted into the bile juice by the protein, glutathione S-transferase, in the liver.(24) ICG is non toxic and non-ionising and its excitation and emission are within 'tissue optical window' thus allowing the visualisation of deeper tissues.(25)

ICG binds with albumin on-specifically and produces singlet oxygen, which is binds to blood proteins. This makes ICG, non-toxic and also prevents its decomposition in the blood.(24)

When ICG is bound with albumin, its excitation and emission occur at a higher wavelength.(26)

Dosage of ICG:

ICG was used on the basis of its dark green colour, at the dose of 25mg/dl for retinal and choroidal angiography.(27) It was administered at a dose of 0.5mg/kg to assess the hepatic function and 2 mg/kg to determine the cardiac output.(28)(29)

Adverse effects of ICG:

There were few reports of urticaria, nausea, dyspnea, peripheral vasodilation and severe anaphylaxis at the doses ranging from 0.5mg/kg to 5mg/kg.(30)

Near infrared imaging:

NIR fluorescence imaging involves injection of a fluorophore(ICG), which is excited at wavelengths of 780 nm or greater, and has emission at wavelengths of 800 nm or greater. NIR fluorophores can be repeatedly excited by the excitation light, and the time lapse between the excitation and emission is a nano-second.

Each molecule of fluorophore(ICG) can emit upto 100,000,000 photons per second, when compared to radionuclide, which gives only a single photon when it reaches to ground state. NIR fluorescence has higher tissue attenuation and scattering, which enhances good tissue penetration.(31)

The NIR wavelength (>750nm) is the 'tissue window' that is available for deep tissue imaging, without the interference of autoflourescence. Autoflourescence is fluorescence emitted by endogenous fluorophores such as NADPH and flavin coenzymes in lysosomes, mitochondria of the cells, and collagen and elastin in the extracellular matrix. This creates a background noise, which prevents detection of lower concentration of fluorophore in the tissues.

In addition to this, there is leakage of excitation light, through the interference filters used to filter the backscattered excitation light and collect only the emitted signal. Thus the noise due to autofluorescence and excitation light leakage is higher than the emitted fluorescent signal from the fluorophore, which necessitates the use of higher sensitivity device and appropriate interference device and use of a higher amount of fluorophore, to collect afluorescent signal with a good resolution. (27)

Clinical applications of ICG and NIR imaging

Intradermal route:

ICG has been injected intra-dermal route, for the evaluation of lymphedema by Unno et al.(32) to assess dynamic lymphatic flow by Rasmussen(33)and for intraoperative assistance during lymphovenous anastamosis for the treatment of lymphedema by Ogata F et al.(34)

Sevick-Muraca(35) determined the minimum dose of ICG, required for detecting sentinel lymph nodes in breast cancer. Murawa et al, demonstrated that ICG fluorescence has a higher sensitivity to detect sentinel lymph nodes over lymphoscintigraphy(36)

Subcutaneous route:

Kitai et al(37) demonstrated 94% detection rates of sentinel lymph node, using ICG and NIR imaging, in breast cancer(34). ICG was used to detect sentinel lymph nodes in gastric cancer by Miyashiro et al(38), Tajima et al(39), lower rectal cancer by Noura et al(40), skin cancer by Tanaka et al(41), lung cancer by Ito et al(42), vulval cancer(43), prostate cancer(44). ICG was also used to detect chylous fistulas intraoperatively by Kamiya et al(45).

Intravenous route:

ICG fluorescence was used to detect patency of coronary grafts by Rubens et al(46), Taggart et al(47), Sekijima et al(48). It was also used to demonstrate hepatic blood flow and to visualise liver segments(49), and to identify lesions of hepatocellular carcinoma intraoperatively and to facilitate precise excision of the same(50).

In Vascular surgery, ICG fluorescence was used to assess graft patency(51), visualise splanchnic circulation and diagnose peripheral arterial occlusive disease and critical limb ischemia(52). ICG due to its lipophilic properties can be used to localise atheromas and efficacy of foam sclerotherapy.(53)

In neurosurgery, ICG fluorescence was found to be superior to conventional angiography to assess patency of vessels in micro-anastomosis in re-vascularisation bypass surgery, aneurysm remnants, stenosis or occlusions in the vessels(54).

In reconstructive microsurgery, ICG was used to identify the perforators and assess the patency of microanastomosis. (51). It is also used to visualise the renal vasculature after kidney transplant(55) and also to assess muscle perfusion(56).

Choosing an appropriate NIR imaging device:

In our background search, we found that, there were no near infrared imaging devices available for clinical use, in our country. The infrared imaging devices that are available in other countries such as Germany and Japan are very expensive and elaborate. Photodynamic Eye (PDE) Hamamatsu,

SPY (Novadaq),

FDPM imager (Texas),

IC-View (Pulsion Medical),

FLARE (Israel Beth Deaconess Hospital),

Custom system (Kochi Medical School) are the six investigational devices used in various published studies. These devices are elaborate and very expensive for the Indian setting.

The cost of the hand-held Photodynamic Eye device is approximately 23 lakh rupees (33,000 euros)

Developing an in-house NIR imaging system of low cost

Our next challenge was to devise an in-house, cost effective and a handy, near infrared imaging system, based on the principles of fluorescent imaging, for our study and also assess the feasibility of ARM technique using this technology in our study setting.

With the expertise at the Bio-engineering department in our institution we were able to devise a low cost, portable, near infrared imaging system using low end components that were available in the market.

Materials and methods

Preliminary work: Choosing between blue dye and fluorescence imaging techniques.

Lymphedema, secondary to axillary dissection is a major concern for most of our patients who are undergoing surgery for breast cancer. Incidence of lymphedema in patients undergoing the same procedure, is 25.24% at our institute. Axillary reverse mapping seems promising in preventing lymphedema in this group of patients. We wanted to assess the feasibility and safety of this technique, in our setting.

Initially we used methylene blue to map the arm lymphatic channels in few patients using the technique of inter-muscular groove injection described earlier. But we were not able to detect the lymphatic channels or the arm node. As the documented detection rates of arm lymphatic channels and arm lymph nodes using blue dye, in literature were low, we decided to use indocyanine green instead, which is a

fluorescent dye to trace the lymphatic channels, after obtaining expert opinion from Dr. Suzanne Klimberg, Director of the Breast Cancer Program, Rockefeller Cancer Institute.

Methylene blue caused skin tattooing, pain and induration at the injection site, and the detection rates with methylene blue were reported to be only 61%.(57) Indocyanine green dye(ICG) was chosen above methylene blue as the identification rates of ARM were 88% (58).

Indocyanine green is a fluorescent dye. Its excitation and emission wavelengths are in between 765 nm - 800 nm and 810 nm - 850 nm respectively and it requires a near infrared imaging device to detect the fluorescent signal and to identify the lymphatic channels and lymph nodes in operating field. In our background search, we found that, there were no near infrared imaging devices available for clinical use, in our country. The infrared imaging devices that are available in other countries such as Germany and Japan are very expensive and elaborate.

Our next challenge was to devise an in-house, cost effective and a handy, near infrared imaging system, based on the principles of fluorescent imaging, for our study and also assess the feasibility of ARM technique using this technology in our study setting.

At the Bio-engineering department in our institution we were able to devise a low cost, portable, near infrared imaging system using low end components that were available in the market.

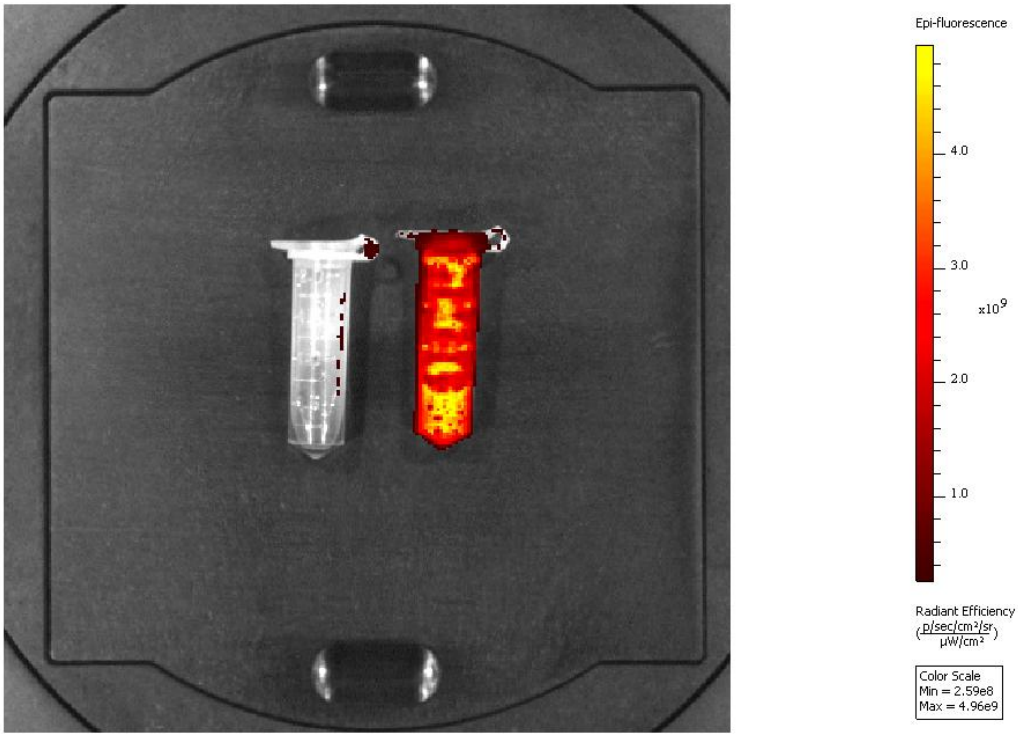
ICG, when used alone, had a weak fluorescent signal in the tissues. It has a short half life and binds nonspecifically to lipoproteins in plasma and lymphatic channels (3). Using our prototype imaging system, we could not appreciate a fluorescent signal, when ICG was excited with 745 nm NIR light source, in our lab. In our further literature search, to find a solution the problem, we found that ICG when bound to albumin (Bovine serum albumin and 20% Human Serum albumin) had higher fluorescence intensity, compared to ICG alone. (4,5).

We had demonstrated this, using Bovine serum albumin (BSA) and Fluorescent imaging system [Camera System Info: Andor Newton; Camera Type: Andor, iKon; Camera CCD Type: DZ436) at Stem Cell Centre, Christian Medical College, Vellore.]

- ICG+ albumin : Excitation at 745nm;

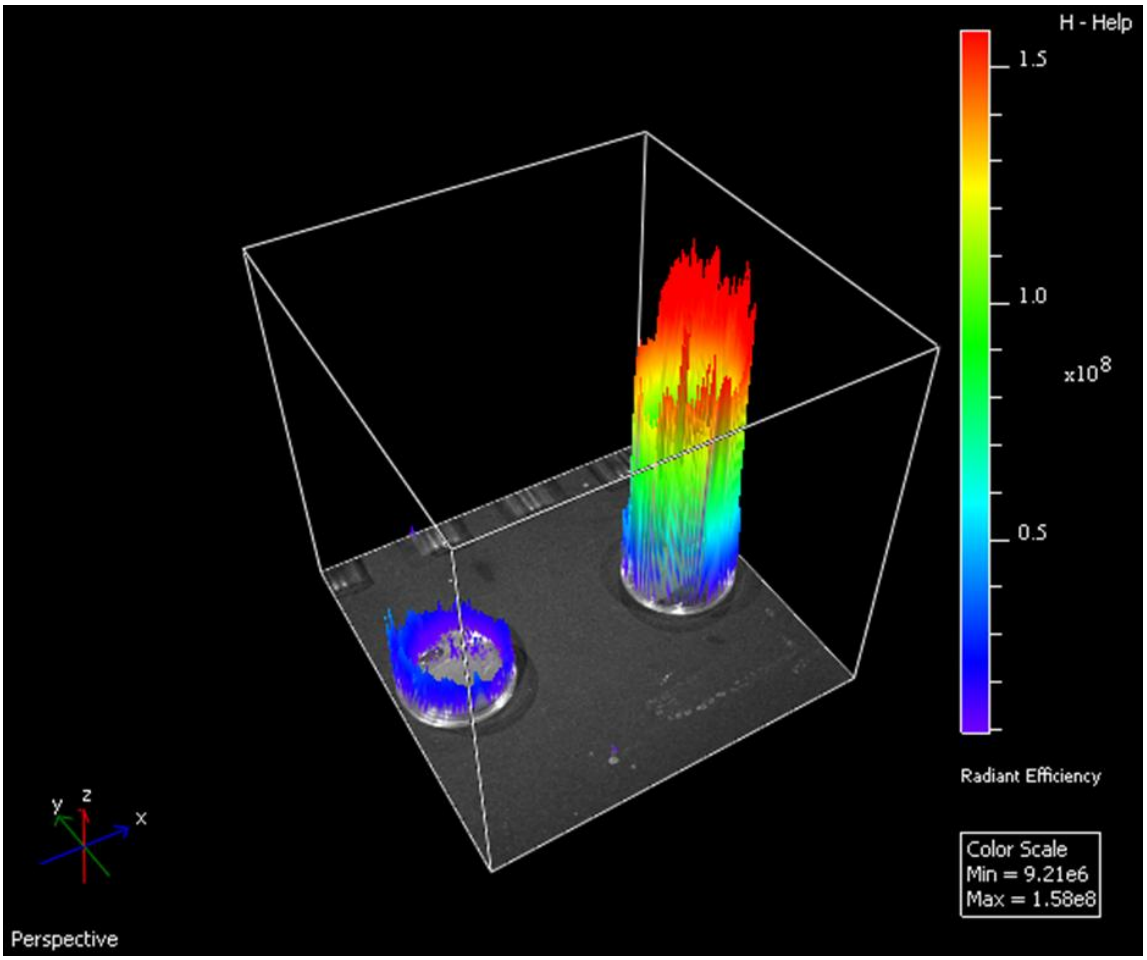
  Emission at 840 nm.

ICG alone (left) ICG+ BSA (right)



Fluorescence intensity map

ICG alone ICG + BSA



The optimal ICG concentration for maximal fluorescence yield within the dermal layer was determined by dissolving various concentrations of ICG ranging from 0.1 to 1 mg/ml in bovine serum albumin (BSA), with concentration of 60g/l.

Bovine serum albumin is antigenic to the human body and causes systemic anaphylaxis. Human Serum albumin (HSA) is also known to cause anaphylactoid reactions and also severe anaphylaxis and there is a theoretical risk of transmission of viral diseases and there are no trials to prove its safety in humans.

Innovating an ICG dye – patient serum combination to increase intensity of signal:

We first explored the use of protein binding to increase signal intensity. However porcine derived albumin described in studies had the limitation of availability, cost and allergenecity. It seemed a natural solution to use the patient's own serum to combine with the ICG dye, Using this technique we found that the high signal intensity produced could be see very well with our low cost device thus setting the stage for the clinical study.

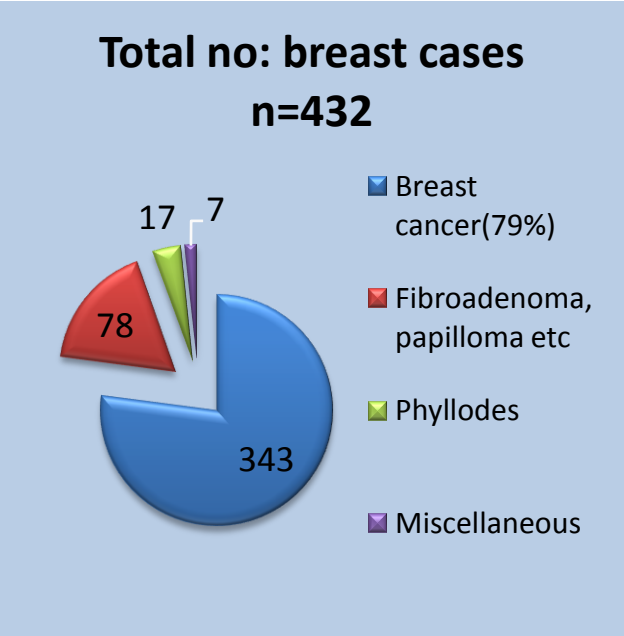
In-house Near infrared imaging system :

In-house near infrared imaging system and optimal concentration of ICG and human serum (patent application is under review)

Working examples

ICG +albumin in a vial (fluorescence seen using the in-house NIR imaging system

Annual in-patient statistics of patients with breast disease in the Department of Endocrine Surgery, CMC Vellore, in the year 2013.



Out of 432 in-patients with breast disorders, 343 patients (79%) had breast cancer.

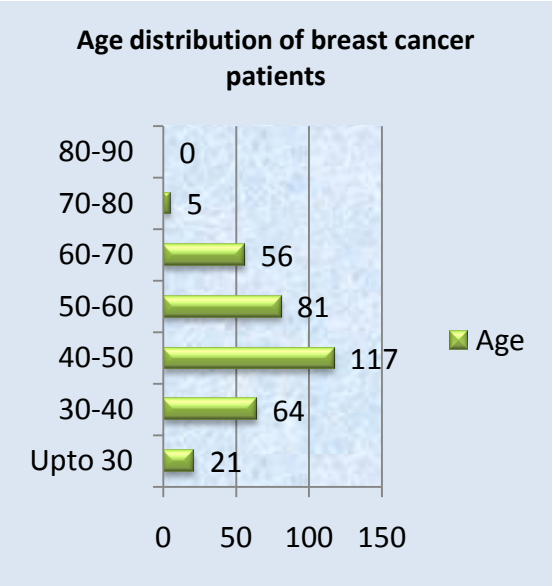


Fig 2. Age distribution of patients with breast cancer treated as in-patients in the Department of Endocrine surgery, CMC Vellore in the year 2013

Majority of patients with breast cancer belonged to the age group of 40-60 years.

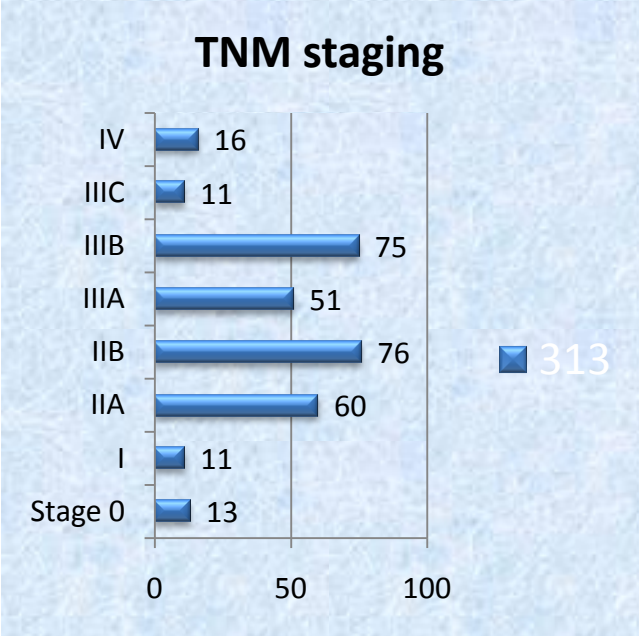


Fig 3 TNM stage distribution of patients with breast cancer treated in the year 2013.

- Patients with Early breast cancer : 51%
- Patients with Locally advanced breast cancer : 43%

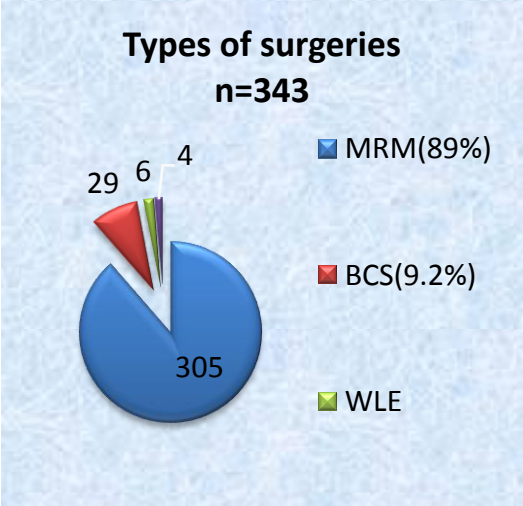


Fig 4. Types of surgeries performed for breast cancer based on the TNM staging of breast cancer in the Department of Endocrine Surgery, CMC Vellore.

Total of 303 patients underwent modified radical mastectomy (simple mastectomy + axillary lymph node dissection) in Department of Endocrine Surgery at CMC Vellore in the year 2013.

Based on the above statistics, our sample size requirement to conduct our study was feasible.

Sample size calculation:

Sample size:

Two Proportion - Hypothesis Testing - Large Proportion - Equal Allocation

Proportion in group I = .61

Proportion in group II = .88

Risk difference = -0.27

Power(%) = 80

Alpha Error(%) = 5

Side = 2

Required sample size for each arm = 40

Alpha Error(%)	Power(%)	Sample Size(n)
5	80	40

Note: with reference to (1) from Thompson etal (Blue dye) the detection rate was found to be (11/18 ie, 61%)and from Noguchi etal (2)it was (7/8 ie 88%) (Fluorescence) with a power at 80% and alpha error at 5% for a 2 sided test we need to study 40 in each arm.

Statistical methods:

All categorical variables will be analyzed using Chi-square test to find the association with ARM lymph node/lymphatic channel identified Y/N against the demographic variables. Continuous variables will be assessed using two independent sample T test. Frequency and Mean ± SD will be calculated as descriptive statistics.

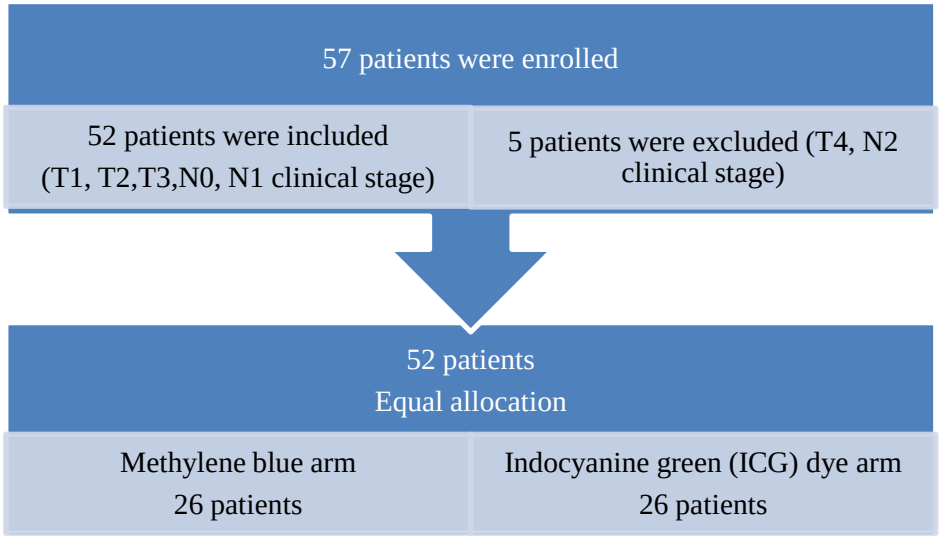
Inclusion criteria:

1. Patients with breast cancer, undergoing axillary lymph node dissection,
2. Clinically N1 stage breast cancer,
3. Patients who underwent neo-adjuvant chemotherapy and hormonal therapy undergoing axillary lymph node dissection,
4. Post-lumpectomy status undergoing axillary lymph node dissection breast conservation surgery with axillary lymph node dissection for early breast cancer.
5. patients who have signed an informed consent form.

Exclusion criteria:

1. Clinically proven N2 and N3 disease,
2. Clinically proven T4a, T4b disease
3. Patients suitable for sentinel lymph node biopsy,
4. Patients who have received RT to axilla,
5. Patients with metastatic breast disease(undergoing palliative mastectomy)

Consort figure:



After standardising the technique of preparation of premixed solution of the ICG and patient's serum and the optimum amount of the dye that is required for injection and site of injection, we were able to detect the lymphatic channel and the lateral group of lymph nodes in 4 patients, who were recruited in the study based on inclusion criteria and after obtaining an informed consent.

As this technique involved various steps in preparation of dye and setting up the imaging system before the operation, it was thought to be inefficient and labour intensive in our setting where there is a shortage of operating time and manpower. In view of this, once the correct site of injection was identified, we returned to using methylene blue dye, which is readily available and could be injected at the same site, to detect the lymphatic channel and lateral group of lymph nodes.

Site of the injection in ARM technique:

Contrary to the usually described inter-muscular groove of the ipsilateral arm, we found that the region posterior to the proximal part of the inter-muscular groove was more accurate in mapping the arm lymphatics. Injection of the dye (both ICG and methylene blue) at this site increased our rates of identification of arm lymphatic channels and lateral group of lymph nodes.

Fig 6. Site of injection of the dye in ARM technique in patients with early breast cancer in Dept of Endocrine Surgery at CMC Vellore. 2014-2015

In our study we want determine and compare the detection rates of the lymphatic channel and lymph node, using premixed solution ICG and patient serum with an in-house imaging system and methylene blue dye and see whether it is feasible to use methylene blue, for mapping the arm lymphatic channel.

The use of blue dye injected in the site identified by ICG has now proved successful. The data of cases using both techniques can be analysed together for assessment of oncologic safety and separately for efficacy in the final analysis.

Results and Discussion:

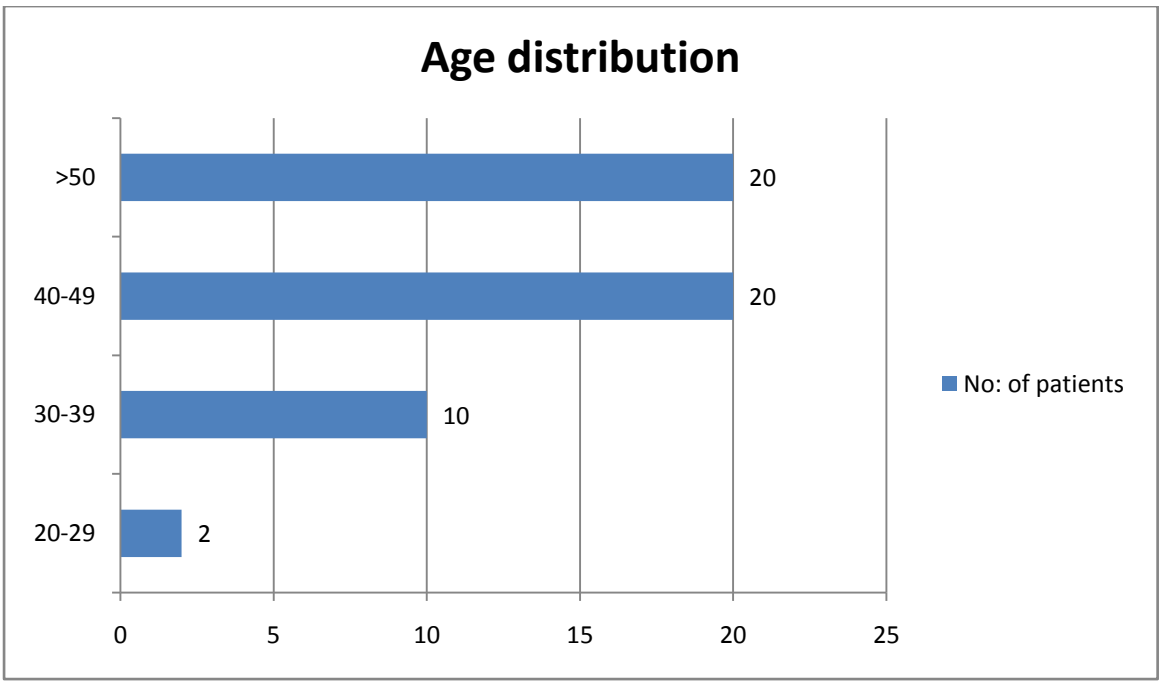


Fig 1. Age distribution of patients with early breast cancer in ARM study in the Dept. Endocrine Surgery at CMC Vellore in 2014-2015.

Majority of patients belonged to the age group of 40-60 years.

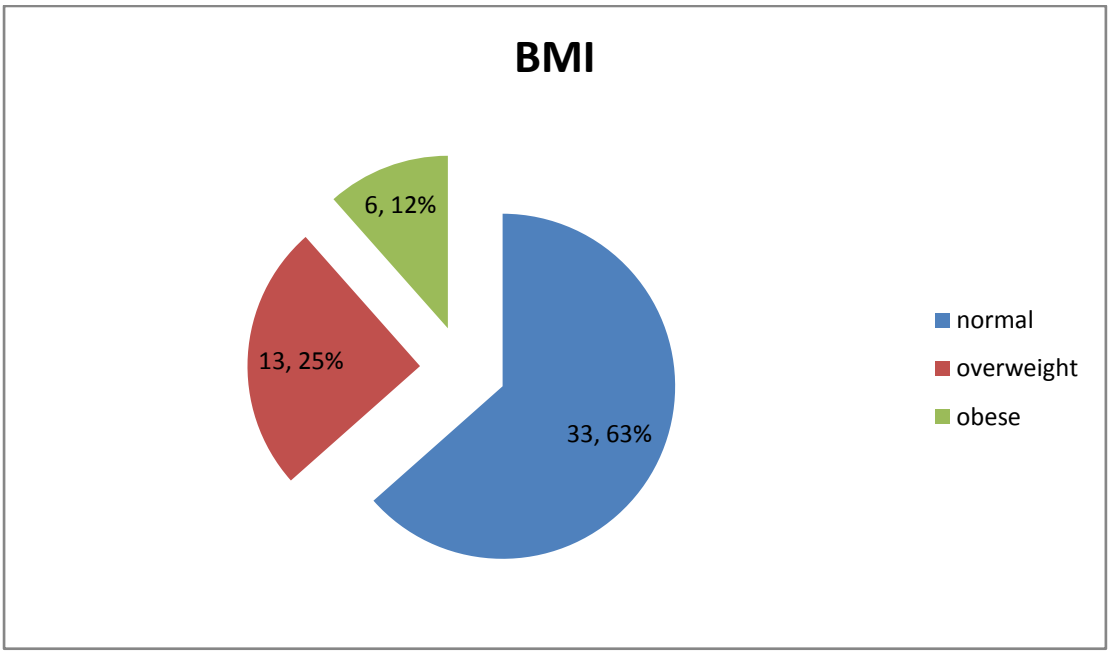


Fig 2. Distribution of BMI of patients with early breast cancer in ARM study in the Dept. Endocrine Surgery, at CMC Vellore in 2014-2015.

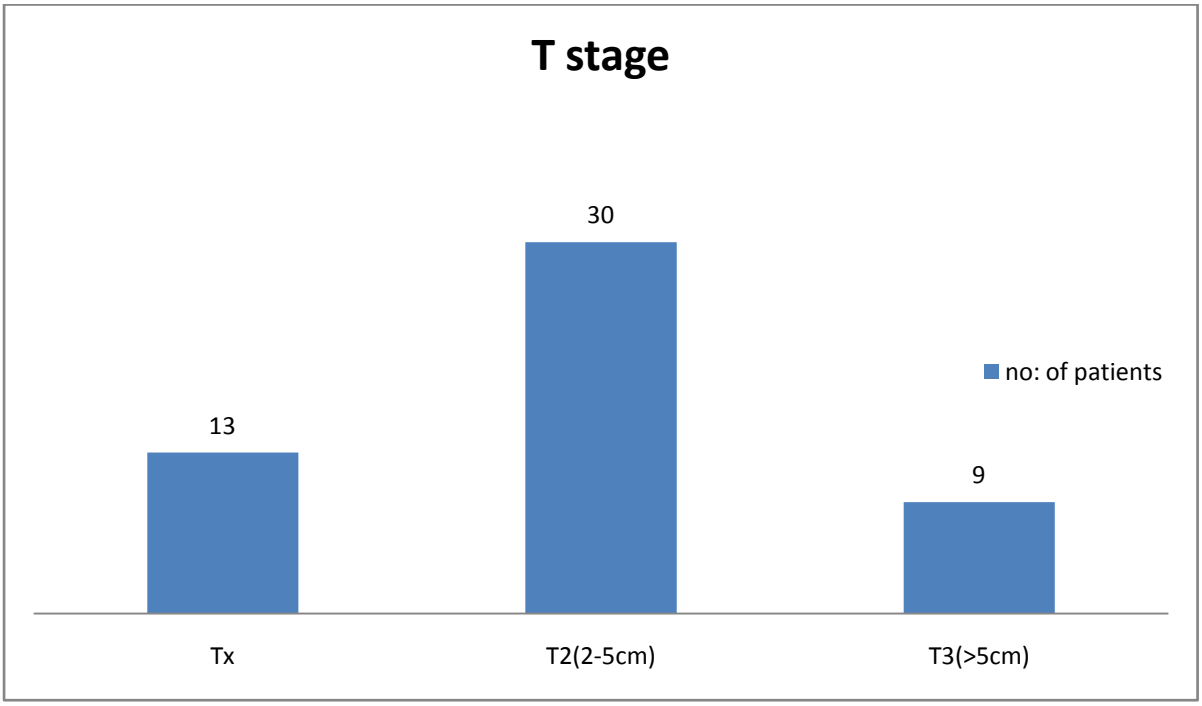


Fig 3. T stage of patients with early breast cancer in ARM study in Dept of Endocrine Surgery, CMC Vellore in 2014-2015.

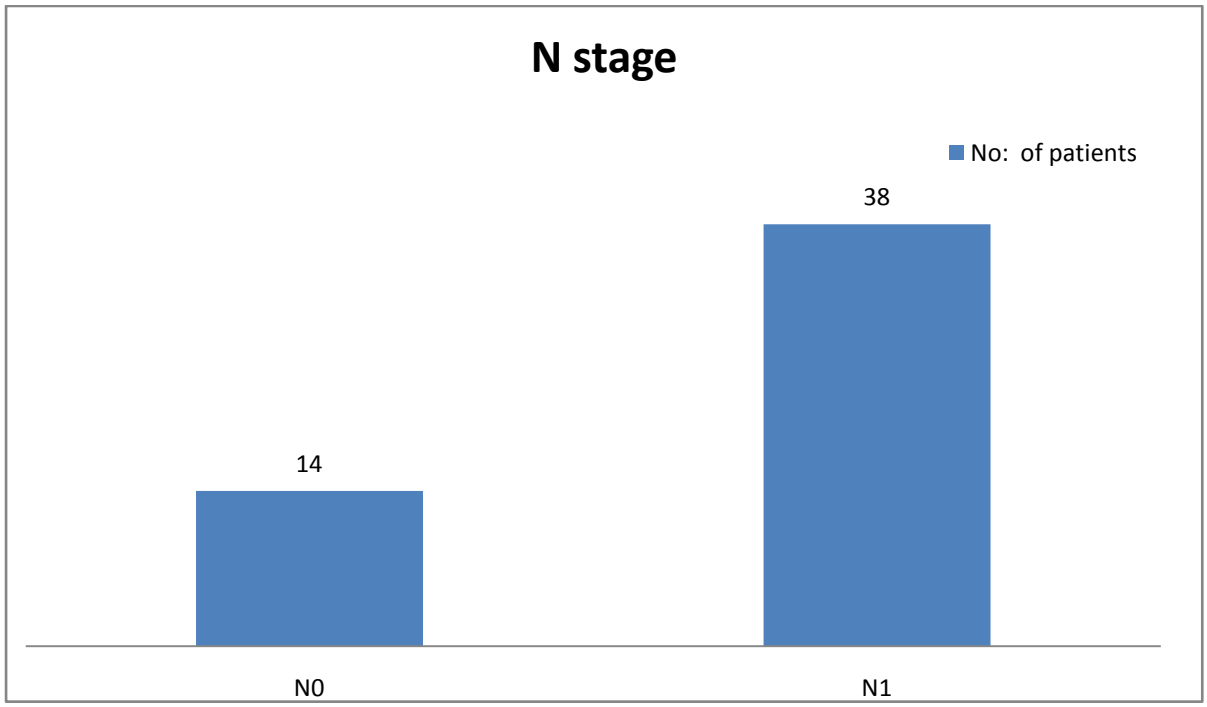


Fig 4. N stage of patients with early breast cancer in ARM study in Dept of Endocrine Surgery, CMC Vellore in 2014-2015.

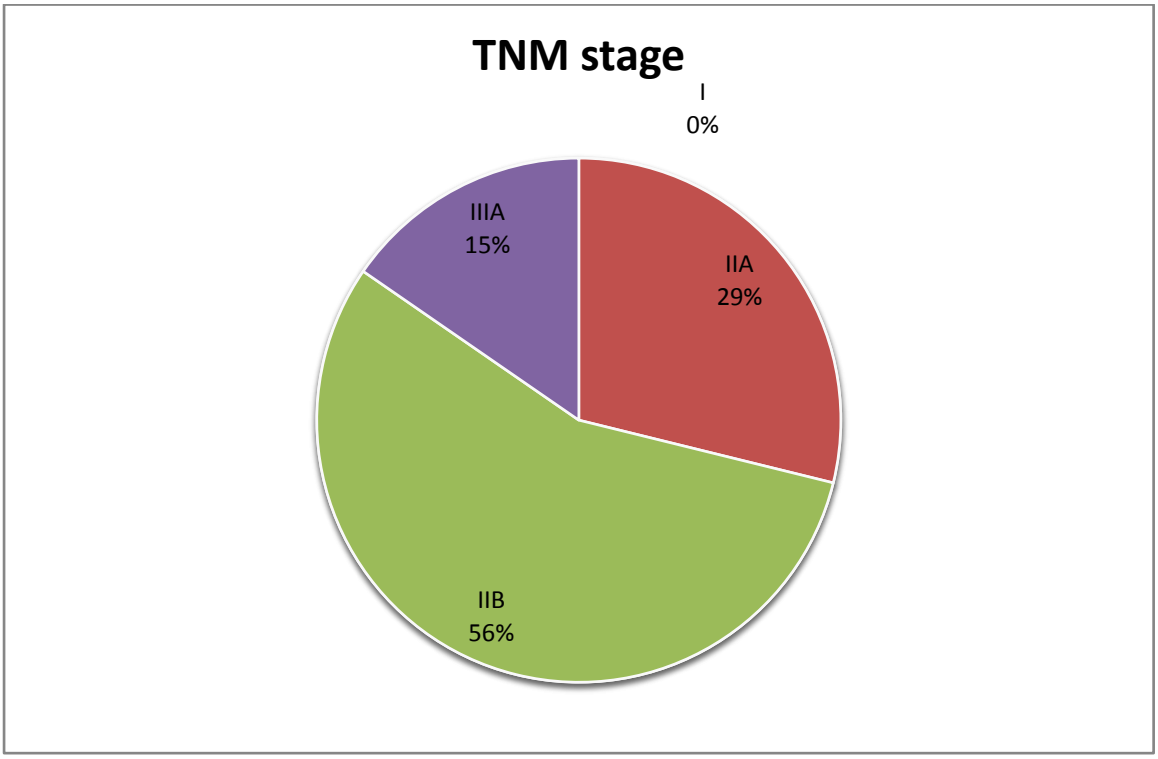


Fig 5. TNM stage distribution of patients with early breast cancer in ARM study conducted in Dept of Endocrine Surgery, at CMC Vellore. 2014-2015

Majority of patients with early breast cancer in the ARM study, belonged to stage IIB of TNM classification of breast cancer.

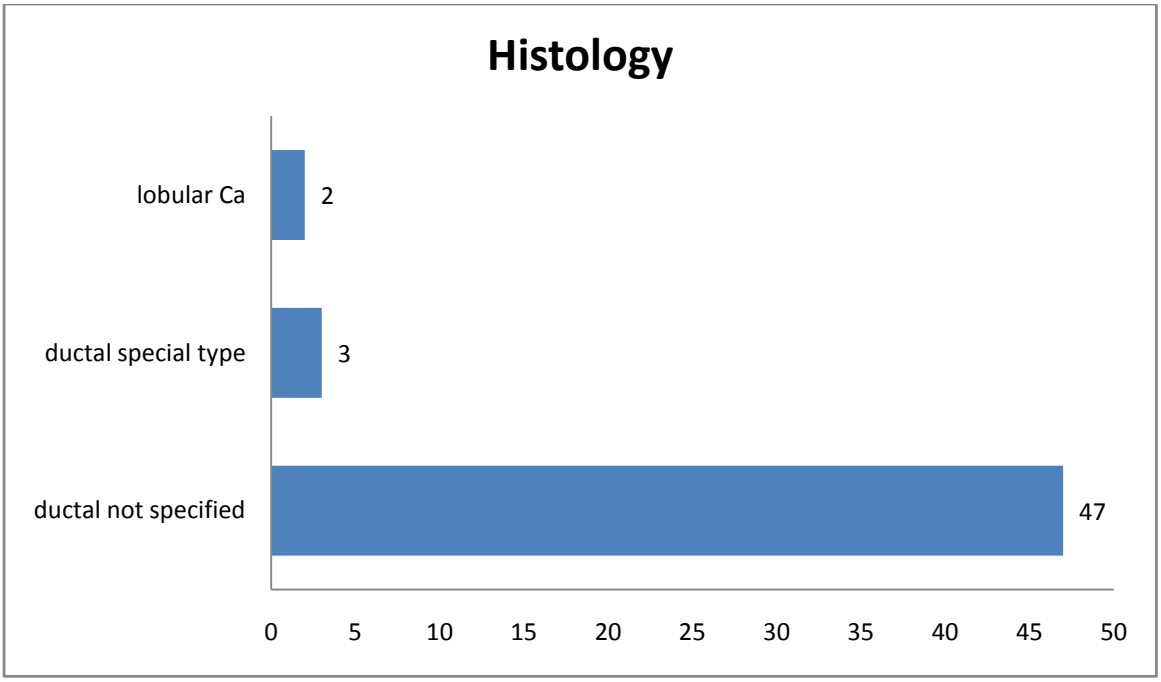


Fig 6. Histology of tumour in patients with early breast cancer enrolled into ARM study in the Dept of Endocrine Surgery at CMC Vellore in 2014-2015.

Ductal carcinoma is the most common type in patients with early breast cancer.

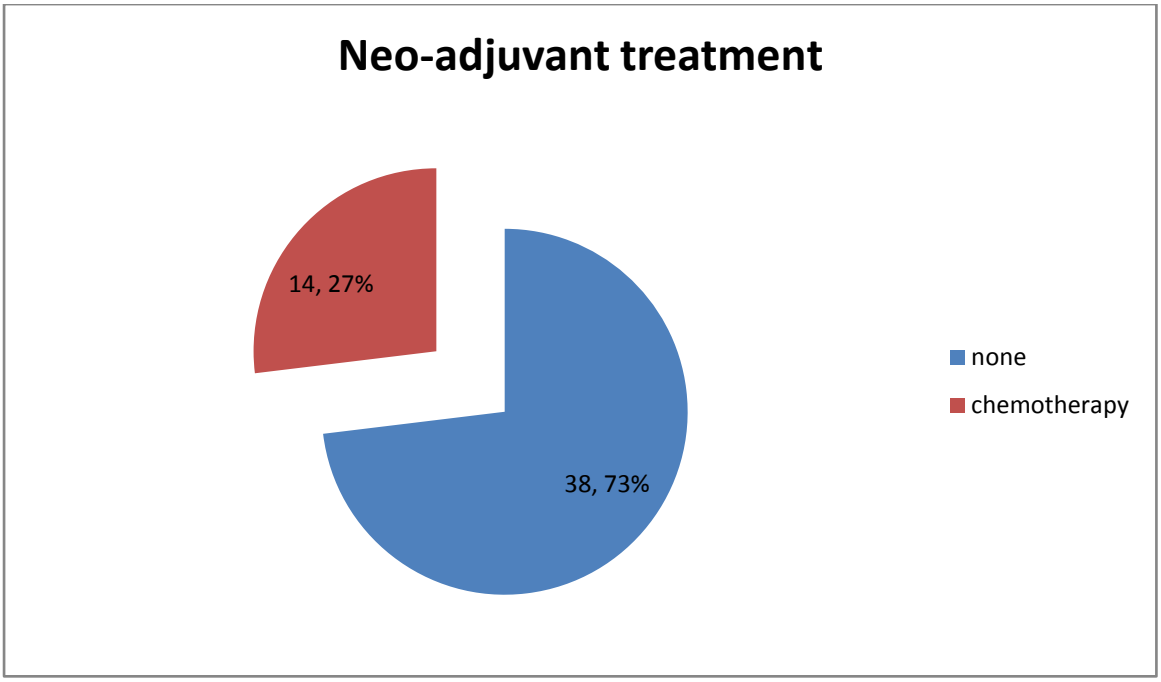


Fig 7. Distribution of patients with early breast cancer, who received neoadjuvant treatment in ARM study in the Dept of Endocrine Surgery, at CMC Vellore.2014-2015

Only 27% of patients with early breast cancer received neoadjuvant chemotherapy.

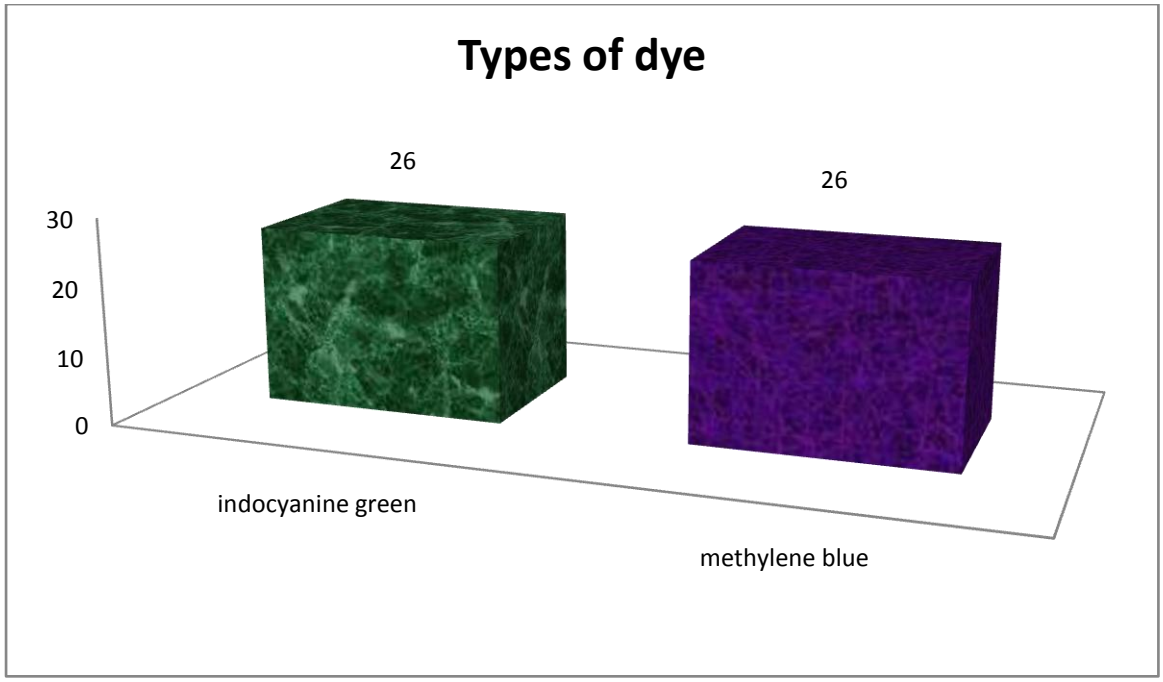
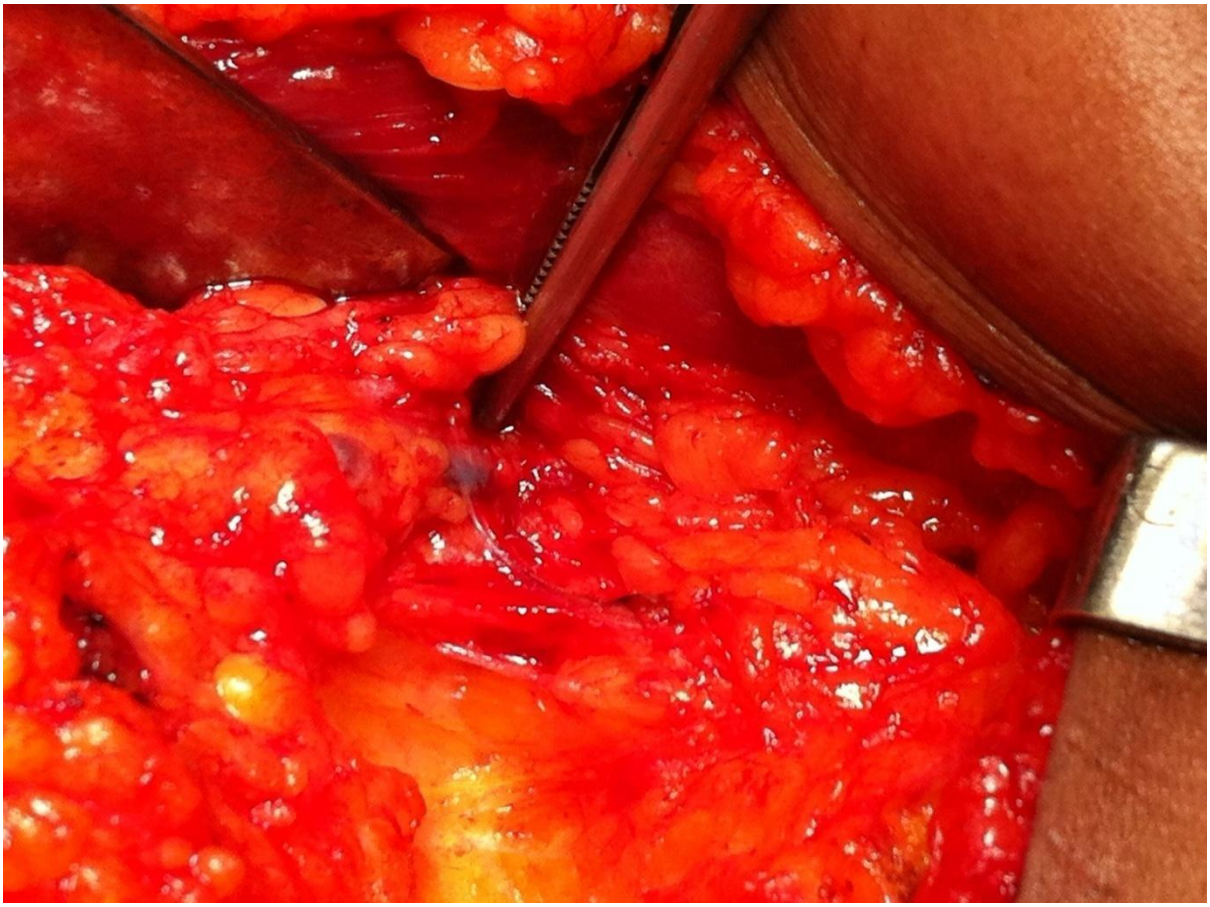


Fig 8 Two types of dye were injected A. Indocyanine green B. Methylene Blue dye in equal proportion of patients with early breast cancer, in the ARM study at CMC Vellore 2014-2015.



Lymphatic channel and arm lymph node detected with methylene blue in patients with early breast cancer, in the ARM study at CMC Vellore 2014-2015.

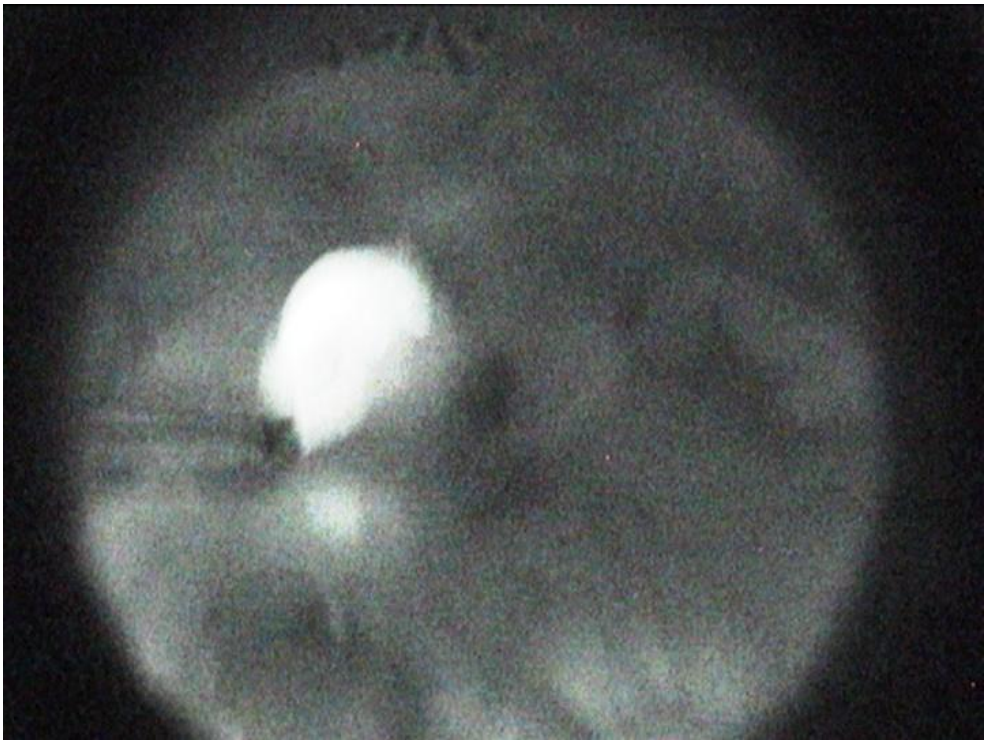


Image showing lymphatic channel and draining lymphnode flowing dermal injection of premixed solution of patient'serum and ICG patients with early breast cancer, in the ARM study at CMC Vellore 2014-2015.

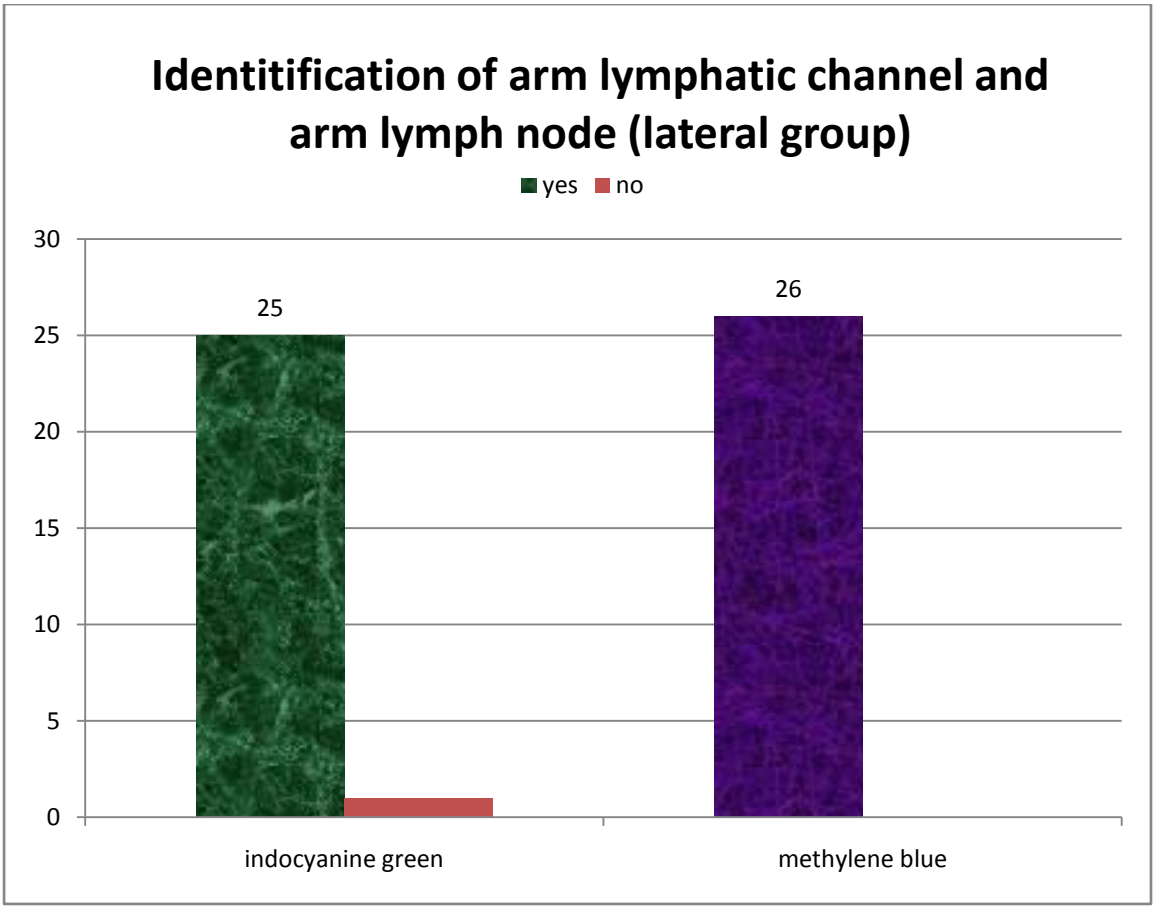


Fig 9. Identification rates of arm lymphatic channel and arm lymph node(lateral group) using indocyanine green and methylene blue in patients with early breast cancer in ARM study in Dept of Endocrine Surgery at CMC Vellore. 2014-2015.

Identification rates of lymphatic channel and lateral group of lymph node is 96.2% using indocyanine green (ICG) dye which is comparable to the identification rates reported in various studies using ICG alone and NIR imaging device.

Identification rate of the lymphatic channel and lateral lymph node using methylene blue is 100% in our study, which is much higher than the reported rate in literature(61%). This is attributed to the identification of the correct site (posterior to the proximal part of the inter-muscular groove) for dye injection in ARM technique to visualise the arm lymphatics.

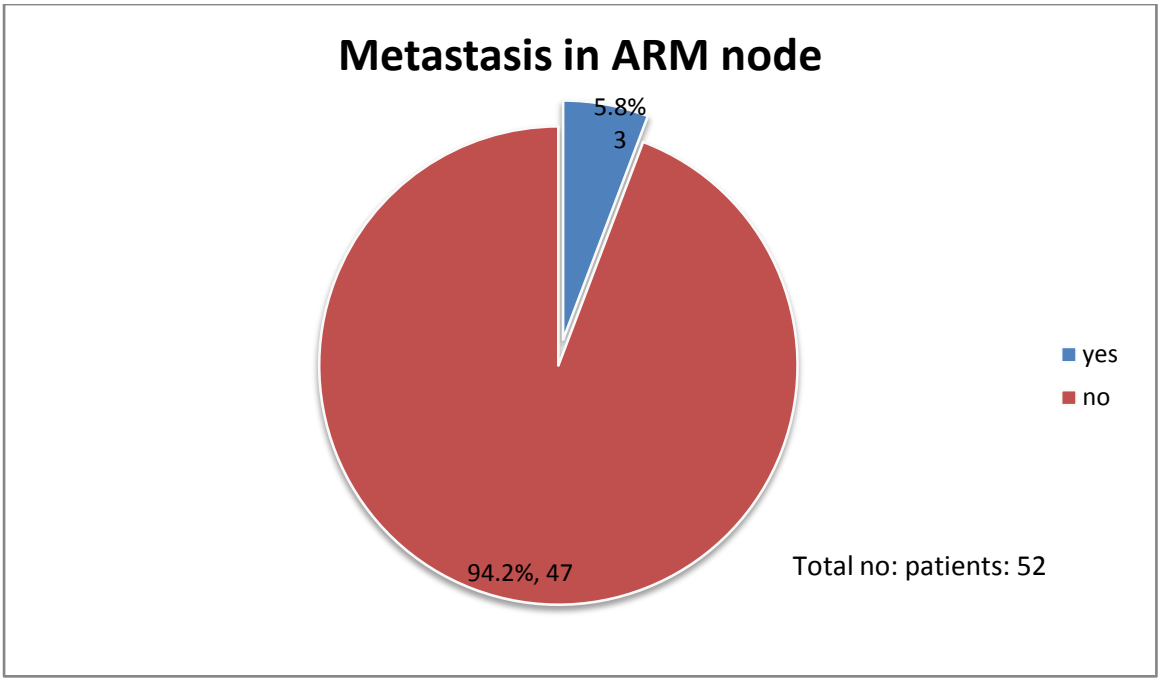


Fig 10. Metastatic rate in the ARM (lateral group) node in patients with early breast cancer in ARM study in Dept of Endocrine Surgery at CMC Vellore in 2014-2015.

Metastatic rate in the lateral group of lymph nodes in our patients with early breast cancer was 5.8%.

As the rate of metastasis in the arm node is low in patients with early breast cancer, once identified the arm lymphatic channel and lateral group of lymph nodes can be preserved, so as to prevent secondary lymphedema, following axillary dissection, thus making the ARM technique oncologically safe.

As the metastatic rate is low, adjuvant chemotherapy or radiotherapy to axilla will be able to eradicate any metastatic tumour deposits in lateral group of lymph nodes, when they are preserved.

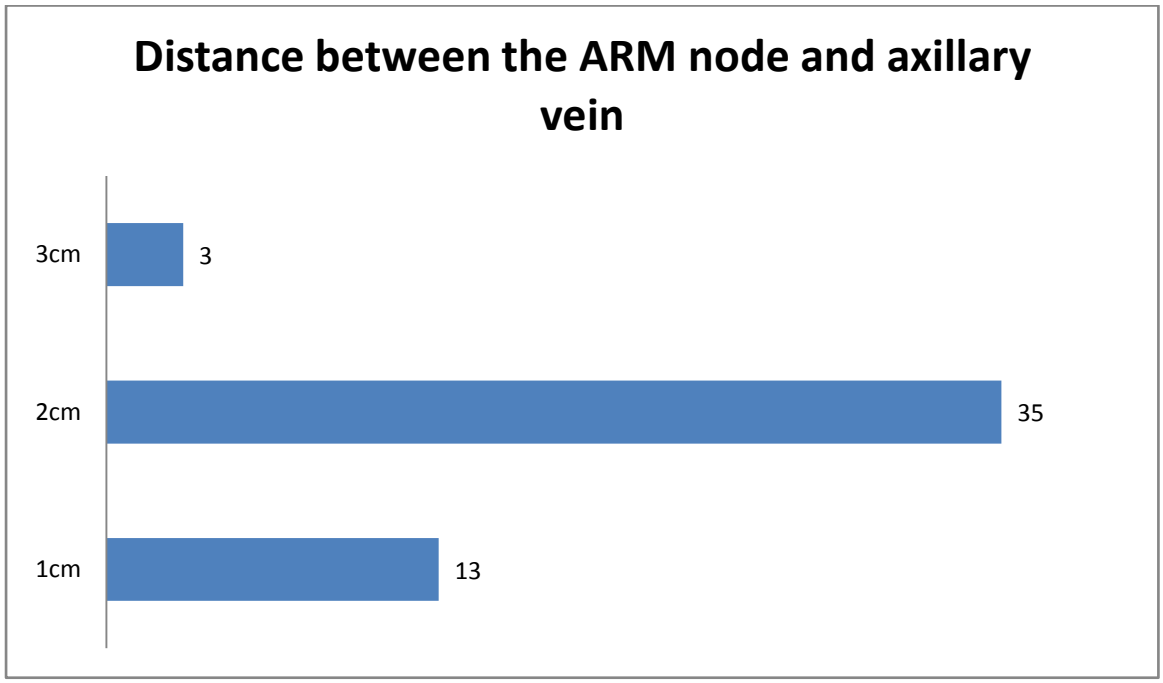


Fig 11. Distance between the ARM node from the axillary vein, in patients with breast cancer, in ARM study conducted in Dept of Endocrine Surgery, in CMC Vellore. 2014-2015.

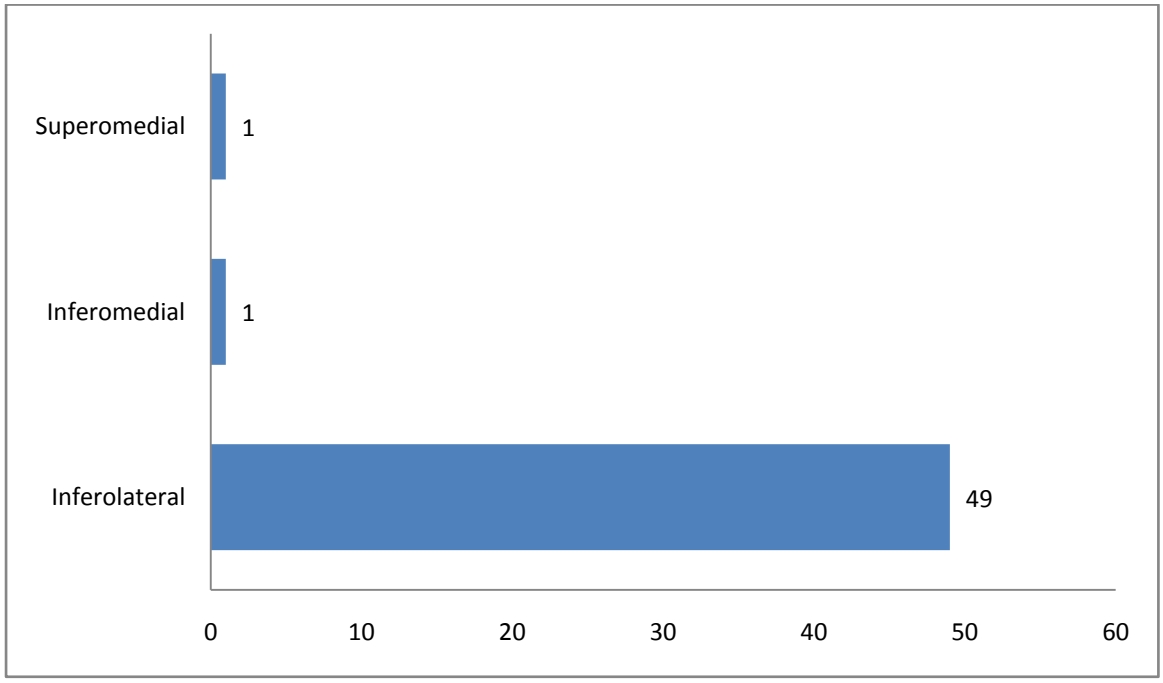


Fig 10 Location of ARM node, in patients with breast cancer in ARM study conducted in Dept of Endocrine Surgery, in CMC Vellore 2014-2015.

The most common position of the lateral group of lymph node was 2 cm from the axillary vein and inferolateral to the thoracodorsal vessels in the axilla.

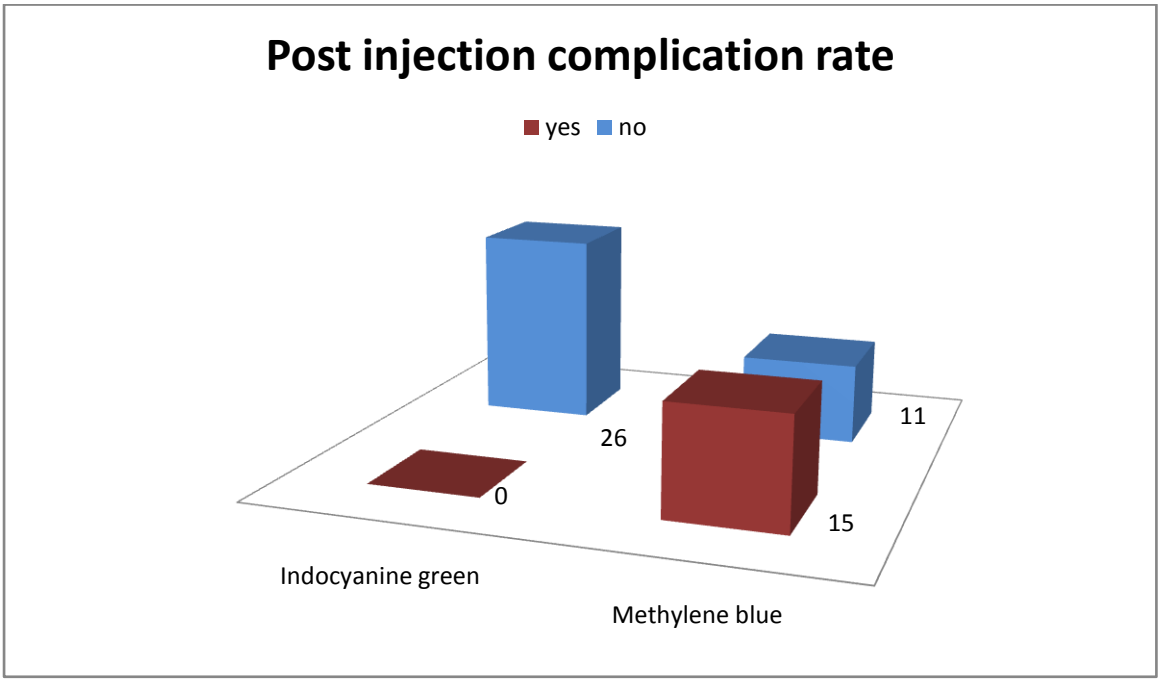


Fig. 10. Rate of complications following injection of methylene blue and Indocyanine green dye in patients with early breast cancer in ARM Study conducted in Dept of Endocrine Surgery at CMC Vellore 2014-2015.

57.7% of patients who were in the methylene blue group developed pain, induration and skin tattooing who were on follow up in OPD for 6-8 days post surgery.

During our study, as we noticed that patients who received methylene blue for reverse mapping, developed pain, induration and skin discolouration. To decrease this morbidity, we further reduced the amount of methylene blue to 2ml and massaged the site of injection longer, with raising the arm to facilitate drainage of the dye into the arm lymphatic channels.

Despite these efforts, the local complications following methylene blue injection were still persistent. This is probably due to the viscosity of methylene blue which does not allow it to readily dissolve in the lymph and may clog the lymphatic channels, disrupting the normal flow of lymph in the arm lymphatic channels, causing local swelling and induration of the arm.

Methylene dye probably could not drain because we were removing the draining lymphatics and nodes in the lateral group to assess the safety of the procedure. In a further study we will need to define the complication rate when the procedure is performed therapeutically and the lymphatics and nodes are preserved which may allow adequate drainage and reduce retention of the dye in the skin.

Amongst these patients, one patient on her follow up for adjuvant chemotherapy complained of persistent swelling at the site of injection. On examination she was found to have induration at that site without any tenderness or skin tattooing.

Limitations

The estimated sample size for our study could be met as some patients refused to consent for the procedure and logistic issues. Only 52 patients could be enrolled into the study and they were equally allocated to the methylene blue group and indocyanine green group with 26 patients in each.

Conclusions

1. Identification rate of arm lymphatics and lateral group of lymph node using indocyanine green dye were 96.2%, which is comparable to the reported identification rate in various studies.

2. There were no complications following the injection of ICG. None of the patients had any pain, swelling or skin tattooing at the site of the injection.

3. Identification rate of lymphatic channel and lateral group of lymph node using methylene blue were 100%, following the identification of the accurate site of injection of the dye in ARM technique.

4. Though the identification rates were high with methylene blue, 57.7% of patients developed local complications following methylene blue, which increased the post-operative morbidity.

5. Metastatic rate in the lateral group of lymph nodes in patients with early breast cancer is only 5.8%.. This low rate of metastasis , early breast cancer, could be treated with adjuvant chemotherapy or radiotherapy, which is already planned as a the course of treatment in these patients. This may allow the lateral group of lymph nodes to be preserved in axillary lymph node dissection, to prevent secondary lymphedema of the ipsilateral arm.

6. Axillary reverse mapping technique is not only feasible but it can also be oncologically safe in patients with early breast cancer.

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Anexures

Consent form

Christian Medical College, Vellore

Department of Endocrine Surgery

Research project : *Axillary Reverse Mapping in breast cancer [ARM study} using premixed solution of Indocyanine green[ICG] dye and autologous serum, and an in-house near infrared imaging system.*

Consent form

Patient name: Age : Sex: Hospital number:

Name of the operation: Date :

Patient

I voluntarily agree to take part in this study.

The doctor named in this form has explained the benefits and risks of the proposed procedure to me.

I agree to the proposed procedure.

I declare that I have read the information sheet provided to me regarding this study and have clarified any doubts that I have had.

I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights.

I understand that I will not receive any other financial compensation.

I understand that my identity will not be relieved in any information released to third parties or published.

I agree to clinical photographs being taken during the course of my stay in hospital. I know that my identity will be protected and that this will be used only for educational purposes.

I know that there will be no intervention done in the treatment of my condition based on the findings; however this study may benefit the patients who are undergoing similar surgery for breast cancer, in future.

Signature of the patient:

Name of the patient: Date:

Witness: 1. _____ (signature) 1._____ (Name in block letters)

Witness: 2. _____(signature) 2._____(Name in block letters)

With regard to the procedure:

I confirm that I have explained the indications, benefits and common risks. I have done this in terms and language which in my judgment are suited to the understanding of the patient.

Name of the doctor:

Signature with Emp no: Date:

Contact details of principal investigator:

Dr. Jyothsna. M,

PG Registrar,

Dept. of General Surgery,

CMC Vellore – 632 004.

Mobile number: 08220313955.

Email id: jyoths03@gmail.com

Christian Medical College, Vellore

Department of Endocrine Surgery

Research project : *Axillary Reverse Mapping in breast cancer [ARM study} using Methylene blue dye.*

Consent form

Patient name: Age : Sex: Hospital number:

Name of the operation: Date :

Patient

I voluntarily agree to take part in this study.

The doctor named in this form has explained the benefits and risks of the proposed procedure to me.

I agree to the proposed procedure.

I declare that I have read the information sheet provided to me regarding this study and have clarified any doubts that I have had.

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I know that there will be no intervention done in the treatment of my condition based on the findings; however this study may benefit the patients who are undergoing similar surgery for breast cancer, in future.

Signature of the patient:

Name of the patient: Date:

Witness: 1. _____ (signature) 1._____ (Name in block letters)

Witness: 2. _____(signature) 2._____(Name in block letters)

With regard to the procedure:

I confirm that I have explained the indications, benefits and common risks. I have done this in terms and language which in my judgment are suited to the understanding of the patient.

Name of the doctor:

Signature with Emp no: Date:

Contact details of principal investigator:

Dr. Jyothsna. M,

PG Registrar,

Dept. of General Surgery,

CMC Vellore – 632 004.

Mobile number: 08220313955.

Email id: jyoths03@gmail.com

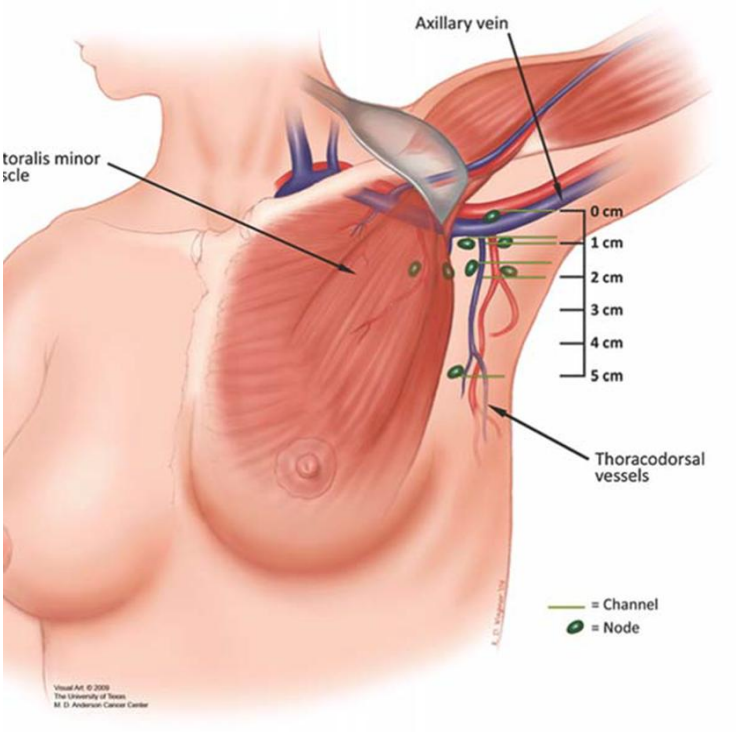
Proforma

DEPARTMENT OF ENDOCRINE SURGERY Date : _____

CHRISTIAN MEDICAL COLLEGE, VELLORE

RESEARCH PROJECT ***: AXILLARY REVERSE MAPPING IN BREAST CANCER [ARM study]***

- Patient Name : Hospital Number: Age :
- Weight : _____ Kg Height :_____cms BMI :____Kg/m2
- Diagnosis :
- Clinical **T** Classification : Tx/T1/ T2 / T3/ T4 Size Of The Tumour: ____cm
- Clinical **N** Classification : N0 /N1/ N2 / N3 Supraclavicular Node: present/absent
- Clinical Stage : I IIa IIb IIIa IIIb IIIc
- Method of Diagnosis of Primary Tumor : FNAC : Y/N; Core Needle Biopsy: Y/N; Excision: Y/N Distant metastasis at diagnosis: present/ absent
- Histology: 1.Ductal not specified
  2. Ductal special type- medullary/colloid/tubular 3. Lobular
  ER: Positive/ Negative Her-2/Neu : Positive/Negative
- Neo-adjuvant Treatment : Chemotherapy/Hormonal/Radiotherapy to axilla/none
- Name of the Surgery:_____ Date of Surgery :_____
- **Arm Procedure** :
- Time of Injection : Time Of Axillary Exposure :
- Time Interval : _____ mins



Location of the ARM node and lymphatic channel

- Lymphatics identified : yes/no -number identified : ___
- ARM lymph node identified : yes/no -number identified : ____
- Distance between the axillary vein to the ARM node : _____cm
- Location of the ARM node in relation to the thoraco-dorsal bundle:
    - Inferolateral / superolateral/inferomedial / superomedial
- **Post procedure**: pain at the site : yes/ no Local reaction: yes/no duration : _____
- Any other complaints : _____
- Metastasis in ARM node : present/absent Ajcc classification:_____
- Post chemotherapy changes in ARM node : present/absent
- Histotype and grade of the tumor: _____
- Peritumoral lymphovascular invasion : present/absent

TNM Staging of carcinoma breast

. TNM Staging System for Breast Cancer

Primary tumor (T)

TX Primary tumor cannot be assessed

T0 No evidence of primary tumor

Tis Carcinoma in situ

Tis (DCIS) : Ductal carcinoma in situ

Tis (LCIS) : Lobular carcinoma in situ

Tis (Paget)

Paget's disease of the nipple with no tumor

Note: Paget's disease associated with a tumor is classified according to the size of the tumor.

T1

Tumor 2 cm in greatest dimension

T1mic Microinvasion 0.1 cm in greatest dimension

T1a Tumor 0.1 cm but not 0.5 cm in greatest dimension

T1b Tumor 0.5 cm but not 1 cm in greatest dimension

T1c Tumor 1 cm but not 2 cm in greatest dimension

T2 Tumor 2 cm but not 5 cm in greatest dimension

T3 Tumor 5 cm in greatest dimension

T4 Tumor of any size with direct extension to

(a) chest wall or

(b) skin, only as described below

T4a Extension to chest wall, not including pectoralis muscle

T4b Edema (including peau d'orange) or ulceration of the skin of the breast, or satellite skin nodules confined to the

same breast

T4c Both T4a and T4b

T4d Inflammatory carcinoma

Regional lymph nodes (N)

NX Regional lymph nodes cannot be assessed (eg, previously removed)

N0 No regional lymph node metastasis

N1 Metastasis in movable ipsilateral axillary lymph node(s)

N2 Metastases in ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent* ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis

N2a Metastasis in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures

N2b Metastasis only in clinically apparent* ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis

N3 Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent* ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement

N3a Metastasis in ipsilateral infraclavicular lymph node(s) and axillary lymph node(s)

N3b Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)

N3c Metastasis in ipsilateral supraclavicular lymph node(s)

Regional lymph nodes (pN)†

pNX Regional lymph nodes cannot be assessed (eg, previously removed or not removed for pathologic study)

pN0 No regional lymph node metastasis histologically, no additional examination for isolated tumor cells‡

pN0(i) No regional lymph node metastasis histologically, negative IHC

pN0(i) No regional lymph node metastasis histologically, positive IHC, no IHC cluster 0.2 mm

pN0(mol) No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)

pN0(mol) No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)

pN1mi Micrometastasis (0.2 mm, none 2.0 mm)

pN1 Metastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent§

pN1a Metastasis in one to three axillary lymph nodes

pN1b Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent§

pN1c Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent§,

pN2 Metastasis in four to nine axillary lymph nodes, or in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis

pN2a Metastasis in four to nine axillary lymph nodes (at least one tumor deposit 2.0 mm)

pN2b Metastasis in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis

TNM Stage Grouping for Breast Cancer

Stage 0

Tis N0 M0

Stage I

T1* N0 M0

Stage IIA

T0 N1 M0

T1* N1 M0

T2 N0 M0

Stage IIB

T2 N1 M0

T3 N0 M0

Stage IIIA

T0 N2 M0

T1* N2 M0

T2 N2 M0

T3 N1 M0 -

T3 N2 M0

Stage IIIB

T4 N0 M0

T4 N1 M0

T4 N2 M0

Stage IIIC

Any T N3 M0

Stage IV

Any T Any N M1